Why do neonatal piglets get diarrhoea?

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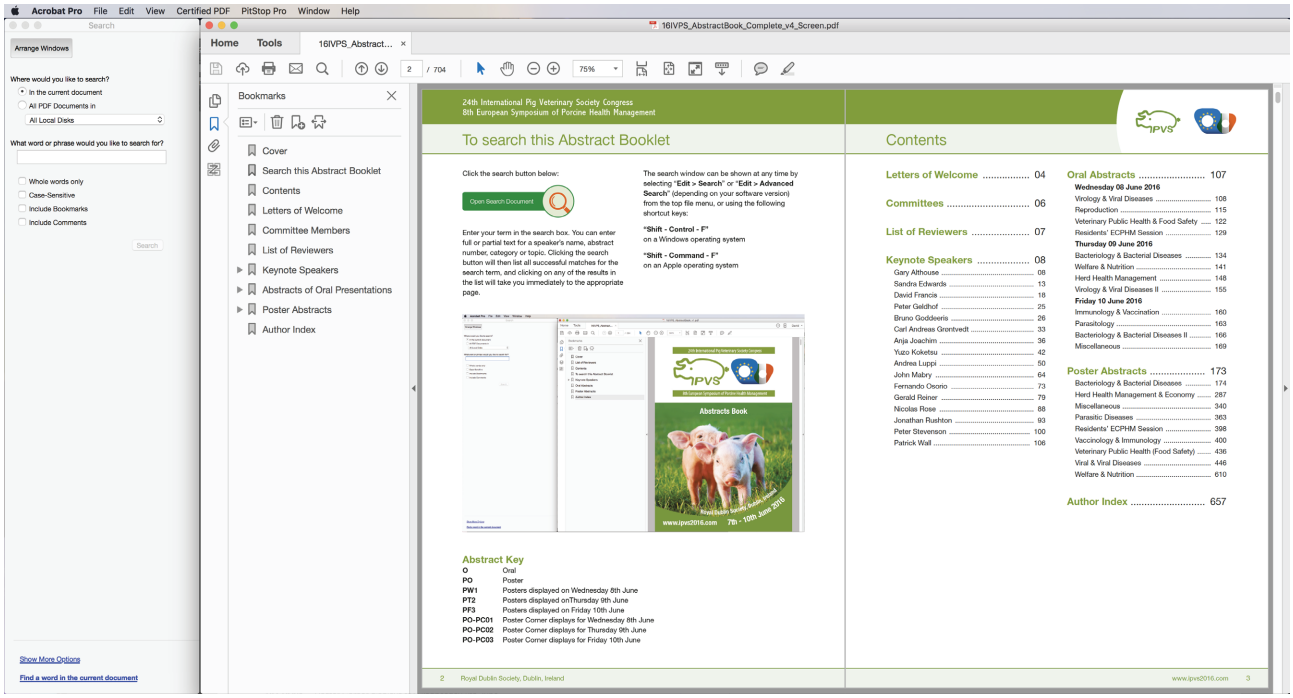

Abstract Key

O	Oral
PO	Poster
PW1	Posters displayed on Wednesday 8th June
PT2	Posters displayed onThursday 9th June
PF3	Posters displayed on Friday 10th June
PO-PC01	Poster Corner displays for Wednesday 8th June
PO-PC02	Poster Corner displays for Thursday 9th June
PO-PC03	Poster Corner displays for Friday 10th June

Contents

Letters of Welcome

As President of this unique collaborative Pig Conference I want to take this opportunity to welcome you, the delegates, to our special and historic event in Dublin. I hope that your attendance in Dublin will forever be remembered with a smile!

In the weeks before we qualified as Veterinary Surgeons in 1989 from University College Dublin we were reminded by an elderly colleague of the verb "to vet" and his interpretation of it as being "to strenuously test / examine and give your seal of approval to". He made the clear distinction between this verb and others associated with the other professions like "to doctor" meaning" to alter or amend", "to nurse" meaning "to care and / or coax along" and "to solicit" meaning……

We as veterinary surgeons / veterinary practitioners / veterinarians are uniquely placed to apply the seal of approval to our endeavours within the pig industry that we serve. This "vetting" is possible through the practical translation of science that we have learned into on-farm meaningful solutions.

This gathering of the international pig veterinary community allows all in attendance to review their veterinary vocation and to assist in the discovery of new solutions to old problems. The contributors to the event have never been busier, delivering over 1100 abstracts for review, ultimately ending in 120 oral presentations and nearly 900 posters. With 3-4 parallel streams, we hope that you will be in a position to take some practical solutions home with you from our gathering. We have designed the programme to provide adequate time for questioning authors, presenters and keynote contributors in the expectation of lively debate and informed discussion. We have built into the programme associated downtime for those in attendance to meet and discuss their problems and propose solutions.

I wish to thank Paolo Martelli and Heiko Natheus for putting together the programme for the joint conference. They have worked tirelessly to complete the programme and to have the best speakers and science possible for your enjoyment over the coming three days. It is my hope that the template that we present here will form the basis of this and future successful pig conferences.

Finally, I would like to acknowledge the secretarial assistance and support of our conference organisers, MCI, and particularly their project manager, Isabella Bottini, without whose help this conference would not have happened.

We hope you enjoy your stay in Ireland and that you will return here often.

Pat Kirwan
President IPVS 2016

Dear Colleagues, dear Readers,

On behalf of the Scientific Committee, I am delighted and honoured to author the preface
On behalf of the Scientific Committee, we are delighted and honoured to author the preface of the proceedings of the 24th IPVS/8th ESPHM. The meeting will be held in Dublin and has been organized under the umbrella of the International Pig Veterinary Society, the European Association of Porcine Health Management, the European College of Porcine Health Management and the Irish Pig Veterinary Society, which is acting as the Local Organizing Committee.

The current edition of the IPVS is unique in that it is the first joint meeting of the IPVS and the European Symposium of Porcine Health Management. The IPVS has a long tradition and is well recognised as the most important world congress in the area of interest, while the ESPHM has a more recent history but, over the years, has quickly evolved into the first major scientific annual event in Europe in the area of porcine health management. The four organizations have decided to join forces to create a successful meeting in Dublin both in terms of participants and scientific content. This collaboration has allowed the scientific approach of the ESPHM to be adapted by the structure of the IPVS. The major changes are a reduction in the number of parallel sessions, an increase in the length of the oral presentations and discussion (15+5 minutes), and a new proceedings format, now based on shorter abstracts rather than one-page extended abstracts. These changes have significantly modified the overall structure of the Congress.

In the current edition, in addition to the traditional "Tom Alexander Memorial Lecture" given by Jill Thomson, 16 main lectures, 116 oral presentations in four parallel sessions and 962 poster presentations (1114 abstracts in total) constitute the core of the congress.

In order to assure a rigorous and fair selection of high quality abstracts to be chosen as oral presentations, each submission was allocated to and reviewed by 2 to 3 reviewers, experts in the specific topic. This review process was double blinded and none of the reviewers were aware of the Authors name(s) or affiliation(s). All reviewers marked the abstracts with 0 to 10 points for scientific quality and 0 to 10 points for impact for practice. Finally, based on the average, each abstract was ranked top to the bottom and allocated into slots for each session according to the topic. The vast majority of submissions, were accepted. Seven abstracts needed revision and 29 were rejected for poor quality. In this way, the International Scientific Committee was able to guarantee the utmost fairness in the selection process.

We wish to express our gratitude to all delegates for their scientific contributions, to the Invited speakers that accepted the invitation to prepare and present an updated review on the topic assigned, to the members of the Scientific Committee for the efforts in selecting the hottest topics to be discussed in the round table discussion at the end of the lectures and the International Review board for the excellent assistance in the reviewing and selection processes. Last but not least, many thanks to the staff that worked so hard in preparing this proceedings book.

We wish the participants a very fruitful and productive congress. We are sure you will leave the IPVS/ESPHM 2016 in Dublin enriched by science and new friendship.

Dublin, 7th of June 2016

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Keynote Speakers

Boar Stud Contributions to Sow Farm Fecundity Goals

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Introduction

The use of high quality boar semen is critical to obtaining desired herd fertility goals. When using artificial insemination (AI) in a breeding program, the semen supplier becomes an external input which can effectively drive a customer's herd reproductive performance. Depending upon circumstance, this input can either aid in or interfere with achieving herd production goals. Abrupt increases in regular return rates leading to repeat services may serve as early indicators of semen quality issues. End-point data suggestive of semen quality problems can include decreases in farrowing rates and average total born per litter, and/or increases in litter scatter. Expectations of fluctuations in this end-point data can be quite variable, driven not just in the etiology of the semen quality issue, but also by herd management factors which may exacerbate the problem. When reviewing the literature, earlier studies (1) have reported mean reductions of 17% and 1.2 piglets born alive due to poor AI semen quality on farms (N=37) experiencing reproductive problems. A more recent retrospective assessment of poor reproductive performance in 600 herds (250-5,000 sows) that use AI identified semen quality issues as the cause of decreased reproductive performance in 120 (33.3%) of the herds (2).

When assessing the impact of a boar stud's contributions to herd fecundity, a process control strategy is employed to identify and eliminate special causes of variation. The diagnostician should obtain both pertinent pre-use data on the extended semen product originating from the stud, along with the post-use fertility data from the sow farm(s). Temporal overlayment of these data often aids in narrowing down the time frame of a more detailed diagnostic focus. Assurances to the accuracy and completeness of information from the stud and sow farm(s) are critical to diagnostic success. This report highlights some of the more recent issues which have been identified as leading to disturbances to semen quality and subsequent herd fertility through recent field investigations.

Initial Ejaculate Quality

All progressive boar studs today employ some type of assessment of a freshly collected ejaculate which is intended for distribution and use in an AI program. The most common ejaculate parameters assessed at a stud include ejaculate volume, color, odor, sperm motility, sperm morphology, and sperm concentration. Ejaculate color/odor, sperm motility and sperm morphology are qualitative assessments, whereas ejaculate volume and sperm concentration data are quantified in order to determine eligible dose numbers from the ejaculate. Minimum requirements of fresh boar semen for use in AI programs have been established (3,4). Color and odor are very quick and simple procedures which are used to identify the presence of certain spermicidal contaminants (i.e., urine, blood, diverticulum fluid). The remaining qualitative parameters of sperm motility and sperm morphology are typically assessed to verify that ejaculates meet or exceed a desired threshold level prior to inclusion in a commercial AI dose. Current recommendations suggest at least ≥80% progressively motile sperm in conjunction with having ≥75% morphologically normal sperm cells (3,4), and are typically defined in a contract between the sow farm and stud. Any investigation should confirm that threshold quality parameters are being met, along with affirming that the stud uniformly applies consistent, objective processes using validated techniques.

Table 1. General stud hygiene and sanitation recommendations (9,10).

Personnel

1. Application of good hand hygiene, including appropriate washing and use of protective gloves, should be practiced throughout all areas of the stud
2. Personnel should avoid contact of bare hands with materials which can later come into contact with the semen or extender
3. Personnel with upper respiratory infections should be cognizant of and avoid contamination of materials, semen or extender through aerosolization during sneezing or coughing
4. Caps/hair nets can be of value if worn by personnel performing the semen collection process and by laboratory personnel as an aid in minimizing hair and dander as a contamination source
5. Clean protective garments and shoes/boots, provided on site by the stud, should be available for use by all stud personnel

Animal housing/handling

1. Animal housing should regularly be sanitized, including removal of organic material and application of a broad-spectrum disinfectant
2. Trimming of hair surrounding the preputial orifice should occur on an as needed basis to eliminate the accumulation of organic matter at this site and its inadvertent introduction into the ejaculate during semen collection
3. The ventral abdomen should be clean and dry prior to commencing with semen collection
4. Cleaning of the preputial opening and surrounding area with a single-use disposable wipe should be considered if the area is wet and/or has organic material present
5. Preputial fluids can contain high numbers of bacteria, therefore, these fluids should be evacuated immediately prior to the semen collection process
6. When collecting semen, the collector should position the penis in such a way as to minimize gravitational contamination of the semen collection vessel with preputial fluids
7. If performing gloved hand semen collection, divert the pre-sperm fraction from the semen collection vessel to reduce ejaculate bacterial load
8. The semen collection area and any collection equipment should be thoroughly cleaned and disinfected at the end of each collection day, with adequate time to dry.

Laboratory

1. Encourage single-use disposable products when economically feasible to minimize cross-contamination
2. When using reusable laboratory materials (i.e. glassware, plasticware, plastic tubing, containers, etc.) which cannot be heat/gas sterilized or boiled, clean products using a laboratory-grade detergent (residue-free) with water, followed by a distilled water rinse, and lastly through a 70% alcohol (non-denatured) rinse. Allow sufficient time and proper ventilation for complete evaporation of residual alcohol. Rinse reusable with semen extender prior to first use
3. Laboratory purified water should be checked on a minimum monthly basis if in-house or by lot if outsourced. Any bacterial growth should be considered significant and appropriate action taken to identify and eliminate the contaminant source
4. Disinfect countertops and contaminated lab equipment at end of processing day with a residue-free detergent and rinse
5. Floor should be mopped at end of day with a disinfectant
6. Break down bulk products into smaller, daily use quantities immediately after opening
7. Ultraviolet lighting can be installed to aid in sanitizing reusables and laboratory surfaces; however, safety precautions should be integrated to prevent exposure to personnel

Keynote Speakers

Table 2. Number of samples to be QC tested (95% CI) relative to the proportion of ejaculates/batches processed at a desired prevalence detection level (11).

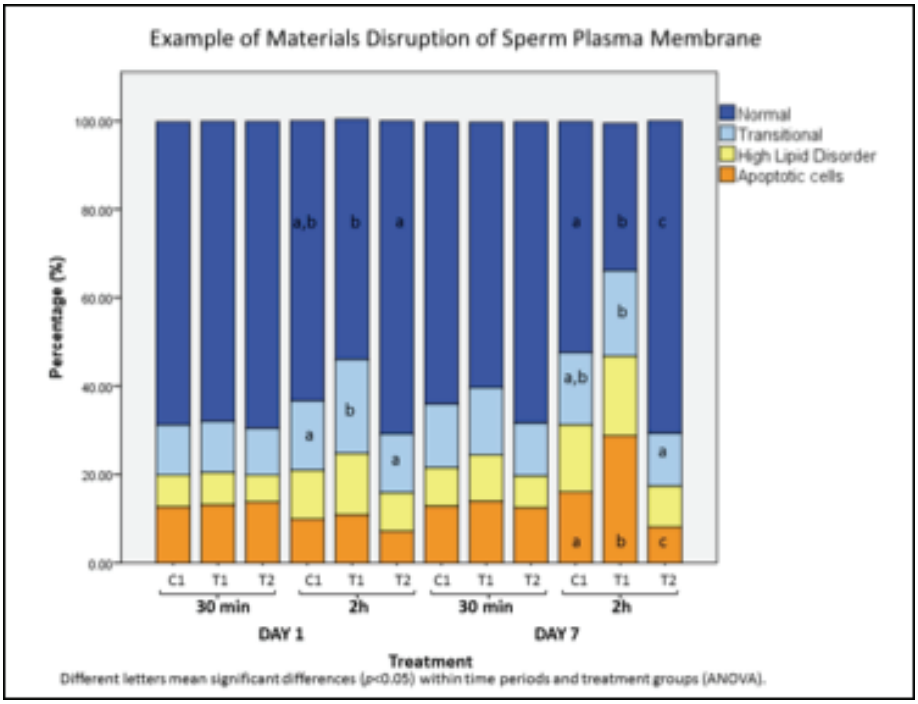
Prevalence Detection Level	Number of batches to be tested (95% CI)				
	Per 100 batches	Per 200 batches	Per 300 batches	Per 400 batches	Per 1000 batches
1%	95	155	189	210	258
5%	44	51	53	54	57
10%	25	27	27	27	28
15%	17	18	18	18	18
20%	13	13	13	13	13
25%	10	10	10	10	10
50%	5	5	5	5	5

Product Contaminants

Adoption of AI in the swine industry has been broad, with this reproductive technology now having a global footprint. As a maturing market, the competition by the suppliers of materials for boar studs remains diverse and intense. Classic marketing strategies by AI materials suppliers have included being a low cost provider or purveyor of quality products. The former drives supplier revenue through sales volume, while the latter achieves revenue through the production of consistent, quality products. In an ideal situation, the optimal materials supplier to a boar stud would be one that combines both cost reduction and product quality improvement. The supplier should be able to demonstrate to the stud acceptable supply chain practices which assure not only consistency and quality of raw ingredients, but also an appropriate level of in vitro and in vivo screening processes on the final product prior to distribution.

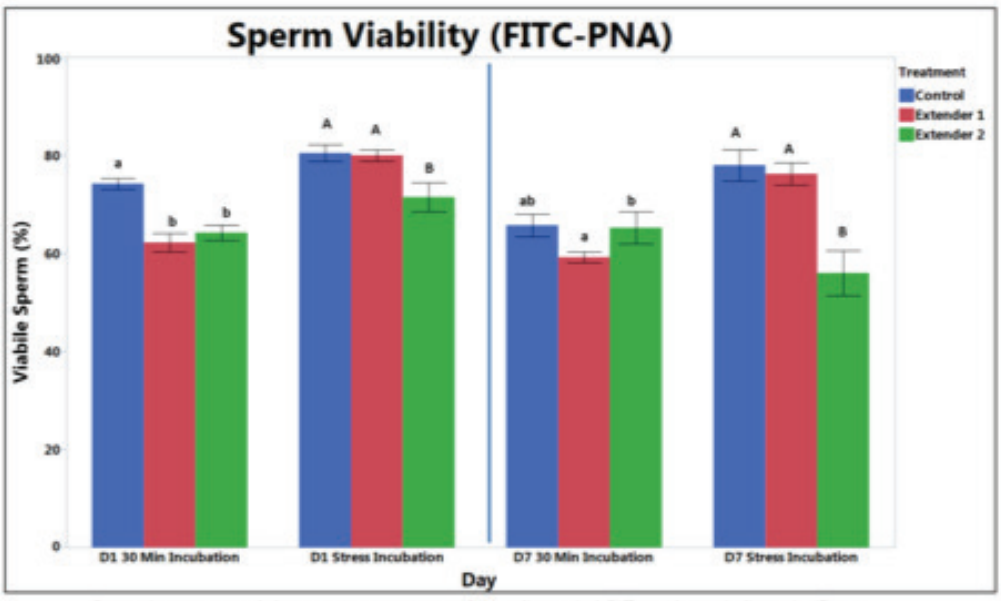
Reports of reduced litter size (total born) across multiple sow farms initiated a field investigation. Multiple (N=3) boar studs sourced the extended semen used on these farms. Sow farm reproductive records were collected and assessed, amounting to ~130,000 services. Of these services, 77% were performed using extended semen in blister bags, with the remaining services using extended semen held in tubes. An initial data review showed distinct fecundity differences based upon whether blister bags or tubes were used for breeding. Retained blister bags and tubes were tested using standard spermiogram analyses (e.g., sperm motility and morphology) over a 7-day storage period, with no differences observed between the receptacles. Subsequently, additional testing was developed in order to assess sperm cell plasma membrane fluidity and applied to samples stored in blister bags and tubes. Time dependent degradation in plasma membrane fluidity was found to occur in association with samples held in the blister bags (Figure 1), suggesting a deleterious effect of material on the sperm. Subsequent work by others (12) identified the leaching of specific compounds (i.e., BPA, BADGE, PVC, phthalates) from the multilayer blister bags which perturbed sperm cells.

Figure 1. Disruption to sperm plasma membrane fluidity associated with exposure to select plastics. Note variation in percentage of sperm exhibiting transitional fluidity between the control (C1), treatment 1 (T1) and 2 (T2) groups.



In 2015, multiple sow farms reported a reduction in litter sizes. Further investigation using data obtained from 36 breeding farms confirmed an average decrease of 0.7 less piglets/litter, starting breeding week 13. Focusing on the time period that reduced litter sizes commenced, the stud's material supply records were assessed, identifying that new semen extender product lots were incorporated into extended semen production. Further in vitro testing of the extender was performed, along with comparison to other semen extenders. Boar semen samples held in the extender in question were found to be stress sensitive, resulting in lower numbers of acrosome intact, viable sperm (Figure 2). Changes in extender choice within the production system lead to a return to normal reproductive performance on the sow farms.

Figure 2. The effect of semen extender on percentages of acrosome-intact, viable (AIV) sperm over time. Under stress incubation, Extender 2 has less (P<0.05) AIV sperm than either currently used extender (Control) or test extender 1.



Keynote Speakers

Conclusion

Semen quality is a vital component to achieving sow herd fecundity goals. Proactively, boar studs need to have protocols in place which objectively assure that the extended semen product meets or exceeds quality parameters and consistency. Boar studs should screen AI material suppliers to assure that they abide by acceptable supply chain practices which support product safety, consistency and traceability. Not only should these practices be able to assure proper design, monitoring, and control of manufacturing processes and facilities, but they should also include an appropriate level of in vitro and in vivo testing which supports quality and consistency of the final product prior to distribution. Detailed recordkeeping of all material/supply lots used at a stud, in conjunction with timely feedback and accessibility to sow farm reproductive records, is crucial to both the stud's quality assurance program and to delineating the contributions the stud has to a sow farm's fecundity goals.

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The perception of pain by pigs and implications for farm and veterinary practice

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Summary

"Freedom from pain, injury and disease" is one of the fundamental aspects of good animal welfare. However, in commercial pig production there are a number of situations where animals may experience pain. This may result from procedures carried out deliberately for management purposes, or from spontaneous health disorders. In order to make decisions on the ethical justification of procedures and the provision of pain alleviation by appropriate anaesthesia and analgesia, it is necessary to assess the intensity and duration of pain experienced by the animals. A number of behavioural, physiological and molecular methods now exist for such assessment but, since pain is a subjective experience which the individual may express in different ways, interpreting these measures can be a challenge. Better methods are required for the practical on-farm assessment of pain and the provision of analgesia when this occurs.

Introduction

The "Five Freedoms" (FAWC, 1993) are used widely as a framework for the assessment of animal welfare and the basis of much legislation for animal protection. Whilst not all Freedoms receive universal agreement, "Freedom from pain, injury and disease" is accepted as important by all stakeholders. Pain can be defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage" (IASP, 1979). Since both sensory and emotional experiences are subjective to the individual concerned, it is not possible to be certain how pain is experienced by a non-verbal species. However, the similarities in anatomical structures and responses to pain which occur in humans and non-human animals, which share common evolutionary functions, suggest that pain in pigs should be given appropriate consideration. It has been suggested that, since pain has an important biological function in learning to avoid actions and situations where tissue damage might occur, total freedom from pain is neither possible nor desirable. However, extreme or sustained unavoidable pain serves no beneficial function for the individual and should be treated as a serious welfare issue.

In the process of breeding and rearing pigs, some animals can experience pain which compromises their welfare. In some cases, this pain results directly from procedures carried out as a result of management decisions. Widespread examples of these include the castration of male pigs to reduce the risk of boar taint in the meat, the docking of piglets' tails to reduce the risk of injurious tail biting in later life, the reduction of canine teeth in neonatal piglets to reduce facial damage from sibling competition, the nose-ringing of outdoor sows to reduce pasture destruction, and the ear tagging, tattooing and slap marking of animals for identification purposes. In other cases, the occurrence of pain is not directly attributable to human intervention, but arises from health disorders developed spontaneously by the animals. These include both acute injuries and infections, and more chronic skeletal, respiratory and enteric conditions. The pain associated with such conditions is still poorly understood, and often relies on human analogy. In this paper, the challenges of understanding, practical assessment and alleviation of pain are discussed with reference to different examples of pain-producing situations, highlighting different issues which must be addressed by scientific research and ethical debate.

Keynote Speakers

The assessment of pain

In a survey of UK veterinarians and pig farmers, many respondents indicated that they found it difficult to assess pain in pigs (Ison and Rutherford, 2014). A number of different approaches exist for the assessment of pain in a practical or experimental context (Rutherford, 2002). The most commonly used approaches relate to the behaviour of the animal. Acute pain is characterised by rapid withdrawal response, escape attempts and by distress vocalisations. The future avoidance of situations in which pain occurred provides evidence that they are associated by the animal with a negative emotional state, rather than the behaviours being simple reflex reactions. Longer term pain can also be assessed by changes in behaviour. These changes can vary from behaviours providing protection to the painful region, such as changes in posture and gait when active, to a state of more general lethargy in which any painful movement is avoided. The extent to which such behavioural changes are reversed when analgesics are administered provides a good indication that they are pain-related, especially if such a response occurs in a dose dependent manner (Weary et al., 2006). Furthermore, the negative affective state can be convincingly demonstrated if animals with supposed painful conditions can be trained to self-administer analgesics, and this can give a method to titrate the extent of the pain.

Since animals have evolved to mask visible signs of pain as an anti-predator adaptation, it is easy to underestimate pain when assessing by behavioural change (Anil et al., 2002). To overcome this, recent methodologies have focussed on more subtle changes. These may be whole body changes in demeanour, as studied by Qualitative Behavioural Assessment (QBA) methods (Wemelsfelder and Lawrence, 2001), or specific changes in facial expression (Grimace Scales), first developed for the study of pain in human infants and subsequently extended to a range of other species including pigs (Di Giminiani et al., 2016a). Because of the masking of spontaneous behavioural signs of pain, an alternative approach is to study evoked responses. The inflammatory responses associated with pain may induce hyperalgesia or allodynia. The heightened sensitivity can be quantified by changes in nociceptive threshold to mechanical or thermal stimulation. This forms the basis of the widely used veterinary diagnostic procedure of palpation of suspected painful areas, but also more controlled experimental use of pressure algometers or lasers (Di Giminiani et al., 2013).

Physiological assessment of pain has often been based on activation of the hypothalamic-pituitary-adrenal axis or of the sympathetic nervous system – the so-called “fight or flight” response. Increased circulating levels of cortisol or adrenaline, or their physiological consequences such as heart, respiration rate or body temperature have been monitored (Lonardi et al., 2015). However, these are somewhat non-specific responses, indicating increased arousal rather than pain per se. While potentially indicative of the extent of a negative affective state in a pain-producing situation, they have a component of fear or anxiety which is present even in the absence of any physical stimulus. Other blood constituents like endorphins or lactate have also been used as pain markers, but none are unambiguous in interpretation. It is known that the relative magnitude of behavioural and physiological responses to welfare challenge may vary between individuals according to the coping strategy they adopt (Koolhaas et al., 1999), which is dependent on both genetic predisposition and early life experience. For this reason, a multifactorial approach to pain assessment involving both behavioural and physiological methods is preferable. However, the behavioural and physiological methods used in the assessment of acute pain may be of little value in assessing persistent but less intense pain. The occurrence of such chronic pain is perhaps the least well understood welfare issue. Newer molecular approaches are helping in the understanding of this (Hunt and Mantyh, 2001), with measurement of changes in expression of genes associated with neural pain pathways now providing evidence of the experience of long term pain.

All of these methodologies are now being applied to current welfare issues within pig production to inform the ethical debate on the acceptability of husbandry practices, to evaluate the efficacy of anaesthesia and analgesia to mitigate the pain associated with such practices if deemed essential, and to assess the desirability of pain relieving interventions in a range of disease states.

Pain associated with husbandry procedures

Castration of male piglets is a pain-causing procedure widely carried out to avoid the risk of undesirable odour and flavour in the meat from compounds associated with male sexual development (the so-called “boar taint”), which markedly reduces the value of the carcass. Whilst animal welfare benefits may also arise, through reduction in aggression and undesirable sexual behaviours as animals reach puberty, it has been shown in experimental studies and large scale practice that intact males can be reared without major welfare problems when management is good. The justification for the procedure can thus be considered as largely for human economic benefit and ethical considerations demand that the need for the practice should be questioned and, if it is considered necessary, that methods to prevent or alleviate any associated pain should be sought. Historically, castration has been carried out on the young piglet without anaesthesia or analgesia. There is no doubt that this procedure causes intense pain during the surgery itself, and that some degree of discomfort persists for several days (von Borell et al., 2009). Given this knowledge, the necessity for the procedure is a topic of current debate. Some countries (UK, Ireland) have largely abolished the practice and other countries are moving in this direction (Backus et al., 2014). This has been stimulated by the 2010 European declaration on alternatives to surgical castration of pigs, a voluntary agreement between stakeholders which states that surgical castration of pigs should be abandoned by 1 January 2018. Whilst progress towards achieving this goal has been made through genetic selection and nutritional interventions to reduce boar taint, and through development of rapid methods for taint detection and on-line carcass sorting, there is uncertainty about how soon these can deliver an acceptable system for entire male production. There are also particular production systems for specialised products, e.g. the heavy pigs for Italian ham production and traditional breeds in organic and silvo-pastoral rearing systems, where use of entire males may still be infeasible. As an interim measure in many countries, and a possible longer term solution in specialist systems, the continuation of surgical castration with prolonged analgesia and/or anaesthesia is being implemented. Whilst injectable analgesia is simpler to apply, and thus the preferred practical option in most countries at the present time, scientific evidence suggests that it is unable to reliably abolish the acute pain experienced during surgery. In several countries, general anaesthesia is, or will soon become, a legal requirement for continuation of surgical castration but the difficulty and cost of this approach make others doubtful about application. The alternative approach of immunological castration is now technically feasible, and implemented in some countries, but gives rise to significant consumer and retailer concerns. Even if these can be overcome, the ethical issue of animal integrity still remains a barrier to any approach except entire male production.

Tail docking is also a deliberate management procedure but, unlike castration, its justification is argued on the basis of a cost/benefit balance for the animal itself. It is carried out on the young piglet to reduce the risk of receiving injury from tail biting in later life, which is unquestionably a serious welfare problem. Tail docking also differs from castration in the degree of pain apparently associated with the procedure, with many farmers believing this to be negligible on the basis of the piglet reactions that they observe. Whilst more detailed scientific study suggests that acute pain does indeed occur (Lonardi et al., 2013), and a difference in expression of Crhr1 mRNA in the amygdala, a molecular marker of anxiety, was detectable at 10 days after neonatal docking (Oberst et al., 2015), other measurements of stress physiology have sometimes shown effects no greater than distress from the handling process (Edwards and Bennett, 2014). However, neuromas which occur as a result of repair processes in injured peripheral nerves in the docked tail (Sandercock et al., 2016a) have been suggested to cause altered peripheral nerve activity that may cause pain or chronic discomfort. Whilst significant uncertainty still exists about this question, it has been shown that, although tails damaged in later life show a prolonged increase in sensitivity, there is no evidence that this is the case following neonatal docking (Di Giminiani et al., 2016b). Furthermore, the molecular markers indicative of changes in peripheral and spinal nociceptive processing associated with possible inflammatory and chronic pain appear to resolve by 4 weeks after tail docking injury (Sandercock et al., 2016b). If it can be demonstrated that long term pain does not occur, and that any acute and medium term pain can be alleviated by appropriate use of anaesthesia and analgesia, then the welfare implications of the procedure for the animal itself might be minimal and justifiable to reduce risk of the far greater harm of being tail bitten. However, this does not remove the ethical argument for respecting the integrity of animals. Some countries have already abolished tail docking and, whilst the prevalence of tail biting is higher than in docked animals, risk can be minimised by appropriate housing and management. The risk factors for tail biting have been widely studied and tools for risk evaluation and risk reduction now exist (Taylor et al., 2012). However, even with

Keynote Speakers

systems deemed to be of low risk, significant tail biting outbreaks can still occur and, in the absence of any reliable method to control their severity once started, many farmers are reluctant to accept such risk. Looking to the future, genetic selection strategies and improvement in enrichment provision offer further risk reduction potential, whilst recent work on the neuroendocrine basis of tail biting may lead to pharmacological control products which could make this reduced risk acceptable in commercial practice.

Even lesser procedures, including ear tagging and ear notching, have now been shown by multidisciplinary assessment methods to cause significant acute pain and merit analgesia (Leslie et al., 2010). Whilst greatest societal and scientific attention has been focussed on the pain associated with deliberate management procedures, from the perspective of the animals these may not be the most important sources of pain-induced welfare compromise. The pain associated with procedures is primarily acute and predictable in time, and therefore amenable to planned pain control interventions. This contrasts with the possibility for both acute and chronic pain associated with unpredictable, and possibly undetected, health conditions.

Pain associated with disease states

The pain associated with lameness arising from trauma, infection or degenerative joint disease is now starting to receive more scientific attention (Jensen et al., 2012). Lameness can be characterised by alteration in gait (Stavrakakis et al., 2015), and these gait alterations can be reduced by administration of analgesics in both sows (Conte et al., 2016) and growing pigs (Meijer et al., 2015) indicating that they are truly reflective of pain. However, the potential for pain associated with other endemic conditions is still relatively neglected. Oesophago-gastric ulcers have been shown in a number of surveys to be widespread in both growing pigs and sows. They are characterised by erosion and ulceration of the lining of the stomach. As they become more severe, intermittent bleeding may take place leading to anaemia and, in extreme cases, massive haemorrhage and death (Friendship, 2006). As many as 60-80% of growing pigs can show some degree of alteration or erosion of the stomach lining, and 5-10% have more serious ulceration. In sows the problem seems even greater, with 25% or more of animals showing ulceration. The extent to which these ulcers cause pain to affected animals in relation to their degree of severity is unknown, although only animals with more severe ulcers show inappetance and loss of condition. In humans, the condition is known to be acutely painful, and the similarity in anatomy might suggest this to also be the case in pigs. If so, the high prevalence of the condition constitutes a serious welfare problem. However, since no reliable diagnostics have been validated in live animals, it is difficult to assess the time course of ulcer development and current degree of severity in order to evaluate the extent of any associated pain and address remediation. Similarly, although pneumonia and pleurisy cause significant chest pain in humans (Kass et al., 2007), a literature search suggests that the extent of this issue in pigs awaits scientific investigation.

Conclusions

The occurrence of pain in pig production compromises animal welfare and must be actively addressed. Where such pain arises from deliberate management decisions, an ethical justification needs to be underpinned by objective scientific assessment of the intensity and duration of pain and distress associated with each course of action. This assessment can be problematic, as our understanding of the subjective experience of pain in animals is still lacking, and multidisciplinary assessment methodologies need to be employed. The assessment of chronic pain is particularly challenging, making it difficult to quantify for endemic health disorders which may have widespread prevalence. Whilst the principal goal must be to remove the sources of pain through modification of production practice and reduction in known risk factors, this will not always be completely effective. A reliable method for on-farm pain assessment is then an essential prerequisite for effective alleviation by appropriate anaesthesia and analgesia, and is a pressing subject for research.

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Keynote Speakers

Mechanism in the Pathogenesis of Colibacillosis - What Can We Do?

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Introduction

Enterotoxigenic Escherichia coli (ETEC) cause diarrheal disease in animals and presumably human beings by first attaching to epithelial cells in the small intestine and thereafter elaborating and delivering one or more enterotoxins to the epithelium. Bacteria may be loosely tethered or more intimately attached to the epithelium, but do not appear to be cytotoxic to those cells. Attachment is by means of fimbriae, which in the case of porcine disease, usually includes either of 4 major fimbrial types:
K88 (also known as F4), K99 (F5), 987P (F6), F18 and F41. Attachment of most of these fimbriae is host-specific for swine. Also, each of these fimbrial types is typically associated with certain E. coli serogroups and correlated with the production of certain enterotoxins (see table 1.). The most common and clinically important enterotoxins produced by these ETEC strains include E. coli heat labile enterotoxin (LT) which is structurally and functionally similar to cholera toxin, and either of two peptide, heat stable toxins: STa and STb. Many strains produce at least two of these three enterotoxins. Strains expressing different fimbriae and enterotoxins may cause clinical disease in different populations of pigs, sorted either by piglet age or swine genotype. Age and genetic selection appears largely due to availability of fimbriae receptors to which the bacteria bind on epithelium.

Table 1: Characteristics of *E. coli* Strains that Cause Diarrhea in Young Pigs

Serogroup	Fimbriae	Toxins	Hemolysins	Age Affected
O8	K99±F41	STa	-	Neonate
O8	K88	LT, STb±STa	+	Neonate & Weaned
O9	K99±F41; 987P	STa	-	Neonate
O20	987P	STa	-	Neonate
O101	K99±F41	STa	-	Neonate
O138	F18ab;ac	STa,STb±Stx2e	+	Weaned
O139	F18ab	STa,STb±Stx2e	+	Weaned
O141	987P	STa	-	Neonate
O141	F18ac	STa,STb±Stx2e	+	Weaned
O149	K88	LT, STb±STa	+	Neonate & Weaned
O157	K88	LT, STb±STa	+	Neonate & Weaned

From: Francis, 2002. J. Swine Health and Prod. 10:171-175.

Strains expressing a particular variant of the K88 fimbriae (K88ac) and also LT plus STb (hereafter called K88 ETEC), are the most frequent causes of ETEC diarrhea, have been the most intensively investigated strains, and are a model for both animal and human ETEC disease. For that reason, they will be the focus of this article. .

The K88 ETEC can cause clinical disease in pigs from birth to several weeks past weaning. Smith and Linggod (1971), using young piglets and K88 ETEC, first established that both toxins and fimbrial adhesins were required for the bacterium to precipitate a diarrheic condition. One year later, Jones and Rutter (1972) reported that when piglets were challenged with K88 ETEC, the bacterium adhered to and colonized porcine intestinal epithelium while K88 negative strains did not. Rutter et al. (1975) subsequently established that piglet susceptibility to K88 ETEC was a trait inherited by pigs as an autosomal dominant condition. Piglets not manifesting that trait, represented as failure of K88 ETEC adherence to isolated small intestinal brush border vesicles, were not susceptible to disease. Thus, it was established in the 1970s, in a K88 ETEC infection model in young piglets, that ETEC causes disease by adhesion to the small intestinal epithelium of the host by means of adhesins (typically fimbriae) and there stimulates diarrhea by means of the enterotoxins produced by those bacteria.

Mechanisms of Pathogenesis Further Investigated.

Further major advances in the basic understanding of ETEC pathogenesis did not occur until the advent of modern molecular technology, when constructed ETEC strains became available. Several investigators, including ourselves examined the roles of individual enterotoxins in the pathogenesis of ETEC. Casey et al. (1998), showed that ETEC expressing STa in the absence of another enterotoxin was able to cause diarrhea. However, these investigators were unable to demonstrate diarrhea when STb alone was expressed. Berberov et al. (2004), Zhang et al. (2006), and Ermue et al. (2008) all demonstrated that LT+ strains of ETEC were highly diarrheogenic to gnotobiotic piglets, and STb+ isogens were moderately to highly diarrheogenic. However, interestingly, both Berberov et al. and Zhang et al. reported that strains expressing LT colonized the porcine intestinal epithelium in significantly greater numbers than did LT-negative isogenic strains, suggesting that LT plays a role in the colonization of the intestinal epithelium by ETEC. However, strains deficient in toxin production, but producing K88 fimbriae did colonize the piglet small intestinal epithelium, usually at a lower concentration, and did not cause diarrhea (Zhang et al.).

Observations made in our laboratory indicated that, when incubated with freshly isolated enterocytes or villi teased from small intestinal specimens, K88 ETEC were unable to adhere. However, if those enterocytes were subjected to osmotic lysis and fractured to produce vesicles of the microvillus surface (brush border vesicles), then incubated with the same bacteria, the bacteria adhered to essentially 100% of the isolated brush borders. From such brush borders, we have isolated a mucin-type glycoprotein (Erickson et al., 1992, 1994; Francis et al., 1998), to which K88 fimbriae adhere, and whose presence is highly correlated with piglet susceptibility to K88 ETEC (Fig. 1, Francis et al., 1998). Thus, this intestinal mucin-type glycoprotein (IMTGP) appears to be a biologically relevant receptor for K88 fimbriae.

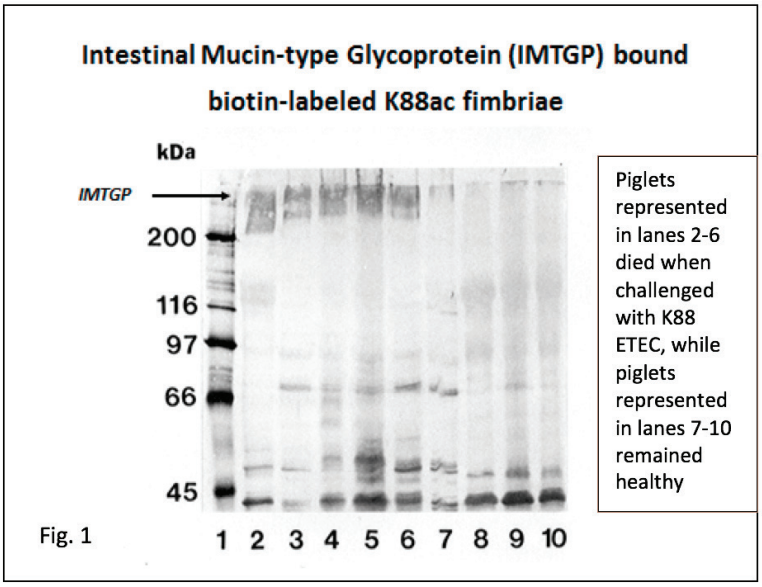
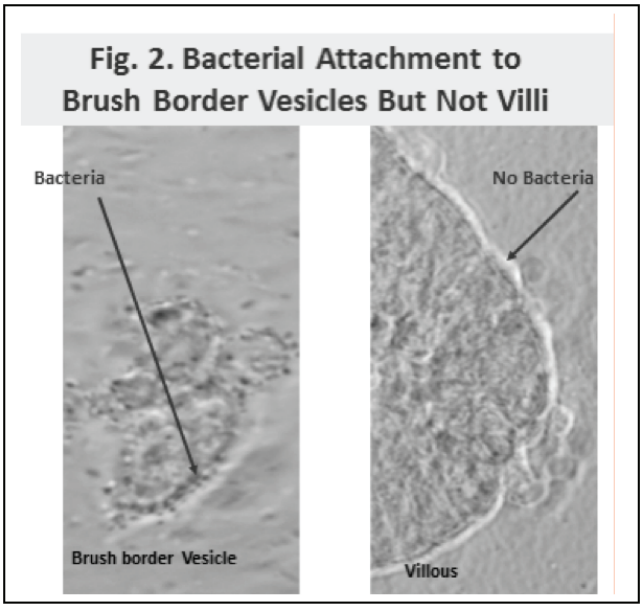


Fig. 1

Co-mingling of villi (clusters of intact epithelial cells) and brush border vesicles from the same animal with K88 ETEC results in saturated attachment of the bacteria to the brush border vesicles, but no bacterial attachment to the villi (Fig 2). This observation suggests that the IMTGP is held cryptic by the enterocyte, but becomes exposed in consequence of cell disruption. While various mucins coat the surface of absorptive intestinal epithelium, it is unlikely that each contains a carbohydrate chain of equal length. We hypothesize that IMTGP represents a shorter mucin structure mingled with longer mucin chains which form a thick glycocalyx, covering IMTGP and blanketing the brush border surface. Electron micrographs prepared by others indicates that the glycocalyx covers only the tips of the microvilli, which on healthy cells are tightly packed. This glycocalyx covering acts as a size-selection sieve, allowing passage of ions and small molecules

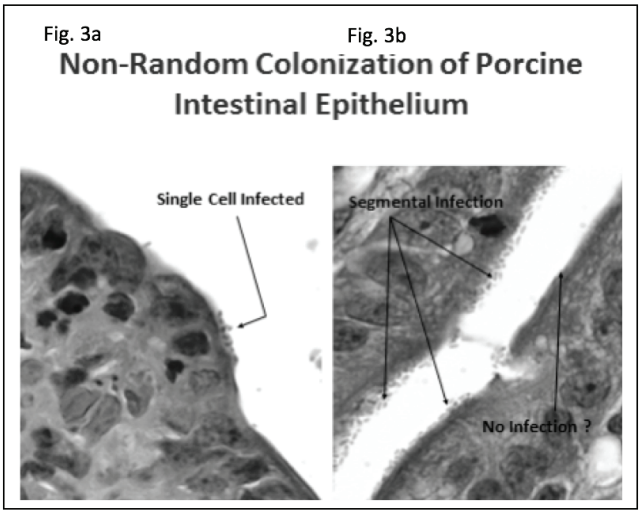


Keynote Speakers

(nutrients), but blocking virus and bacteria from cellular contact (Frey et al., 1996). When enterocytes are osmotically disrupted and cells are broken apart, the tight packing of the microvilli is relaxed, and possibly much of the glycocalyx is lost due to sheer force action associated with the grinding of cells as done in the preparation of brush border vesicles. Perhaps receptors are exposed as glycocalyx is lost on the apical surfaces of the microvilli. The above described cell membrane changes may also expose receptors on the lateral sides of the microvilli on which glycocalyx is not produced. These observations and hypotheses suggest why receptors are exposed on isolated brush borders and not on healthy epithelial cells, but they do not explain how natural infection in young pigs is initiated and propagated. As older pigs are not susceptible to ETEC even though they produce IMTGP throughout life, it is possible that the glycocalyx thickens with age, preventing surface exposure of the IMTGP.

Colonization initiates in a few cells and advances in a mosaic pattern

In preliminary studies, we inoculated porcine small intestine biopsy specimens with high concentrations (approximately 109-1011CFU/ml) of K88 ETEC with which they were incubated for up to 4h. They were then washed for removal of non-adherent bacteria and prepared for histological sectioning and stained for the observation of adhesive bacteria. We found only an occasional epithelial cell to which bacteria had adhered (in most instances with several to many bacteria), suggesting that the remainder of the epithelial cells were refractory to bacterial attachment. We made similar observations in ligated loop experiments in young piglets (Fig 3a). Isolated epithelial cells heavily colonized with bacteria may be observed with surrounding cells unaffected. With more advanced colonization, a mosaic pattern of bacterial adhesion is observed, giving the impression that infection progresses from isolated foci by bacterial outgrowth from cell to neighboring cell, rather than by randomly diffused colonization (Fig 3b). Similar patterns can be observed in tissues from naturally infected animals. Typically, infection exhibits a mosaic pattern. In addition, we have observed a similar mosaic pattern of infection in piglets experimentally infected with enterohemorrhagic E. coli, including strains of serotype O157:H7 (Francis et al., 1986). This observation suggests a common strategy by pathogenic E. coli in colonizing intestinal epithelium.



In summary, the observations of others and ourselves indicate that both adhesive fimbriae and enterotoxins are essential to the development of diarrheal disease in pigs, that specific fimbrial receptors must be present and exposed to the lumen of the intestine for ETEC colonization to occur, and that enterotoxins (at least LT) may contribute in some way to colonization of the intestine as well as to the diarrheal condition that follows colonization. Regarding K88 ETEC, only animals expressing a specific receptor (IMTGP) are susceptible to clinical infection. Further, our research suggests that these specific receptors are held cryptic on most enterocytes, but that colonization on permissive enterocytes, where those receptors are apparently exposed, leads to the exposure of receptors on adjacent cells. As pigs become older, they appear to become refractory to ETEC infection while at least in the case of K88, receptor expression remains strong. Therefore, it appears likely that receptor exposure decreases with piglet age, possibly through thickening of the glycocalyx that covers the absorptive epithelium.

Oral or Intranasal immunization with ETEC results in a robust immune response and protection against clinical post weaning ETEC disease.

Vaccines to be delivered to pregnant sows for the protection of neonatal piglets from ETEC disease have long been widely available and appear to be highly effective. Vaccines for protecting weaned pigs from ETEC strains that affect those animals are not widely available and independent analysis of their efficacy has not been done. However, published research indicates post-weaning vaccines can be highly effective in protecting such animals.

A major challenge in protecting weaned pigs from ETEC is the narrow window of opportunity for immunization given that pigs may be weaned as early as 3 weeks of age and susceptible to infection shortly thereafter. Concerns for the efficacy of such vaccines might include that piglets are immunologically immature at the time a vaccine must be delivered, or that maternal antibodies might interfere with immune development. In our laboratory, we have tested both a constructed live, and a multi-subunit vaccine for efficacy in protecting pigs against postweaning ETEC disease. Both were found highly protective. However, neither has been tested on piglets while those animals were still nursing their dams.

The live vaccine was constructed utilizing an avirulent E. coli strain (G85-1; O101:K26:NM) originally containing no known virulence determinants, but modified by transformation to possess and express the b subunit of LT enterotoxin, and conjugated to possess a plasmid that expressed K88 fimbriae (Francis and Willgohs, 1991). Piglets utilized in the study were naturally farrowed from sows selected from stock predominantly susceptible to K88 ETEC, and suckled for 10 days before being separated from their dams, and placed on milk replacer. Piglets were subsequently given vaccine strains (per os at 14 and 19 days of age), alternatively expressing both virulence antigens (K88 and LTb), either antigen alone, or neither antigen. Following vaccination, all pigs were challenged (at 28 days of age) with a highly virulent ETEC strain (of serogroup O157) expressing K88ac, LT and STb. Thereafter, pigs were observed for disease.

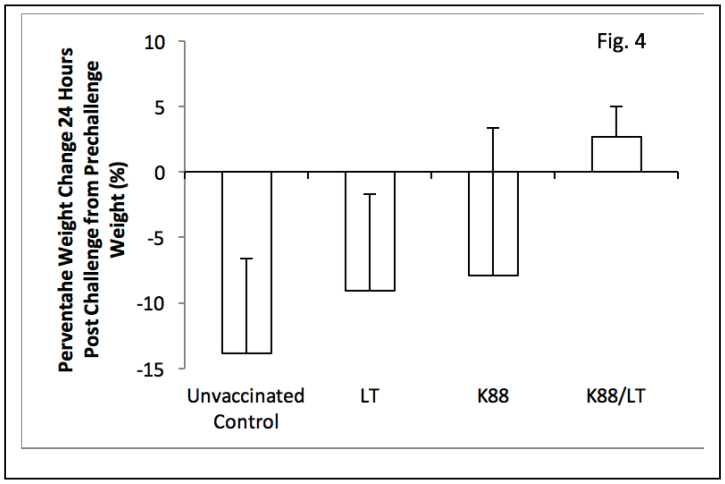
Table 2: Clinical Outcomes following ETEC among Pigs Vaccinated with Constructed Vaccine Strain

Plasmid gene(s)	No. Pigs	Diarrhea	≥ 10% weight loss	Death*
K88/LTb	33	1(3)	0(0)	0(0)
K88	18	5(28)	2(11)	2(11)
LTb	14	14(100)	12(86)	10(71)
None	55	37(67)	23(42)	22(40)

( )=%
*died or were euthanized when moribund

Pigs receiving the vaccine strain expressing both virulence antigens were highly protected from ETEC challenge (Table 2). Partial protection was noted among pigs receiving the vaccine expressing K88 only, but no protection was observed among pigs receiving the vaccine containing only the LTb gene when compared with that of pigs receiving the control vaccine (parent strain without virulence genes). Pigs among controls that did not develop illness were likely of genotypes inherently resistant to K88 ETEC.

Our post-weaning pig vaccine studies employing multi-subunit vaccines were conducted more recently, with more parameters measured that just clinical outcomes. The subunit vaccines consisted of purified K88 antigens and a commercially available preparation of LT toxin, or either antigen alone. Control piglets were inoculated with vaccine diluent only. All piglets were delivered from K88-receptor homozygous sows into germ-free isolators then inoculated with Lactobacillus acidophilus and the non-pathogenic E. coli strain G58-1 mentioned above, to represent an intestinal flora. Piglets were inoculated intranasally with the purified antigens on days 10 and 17 of life and challenged with the same highly virulent ETEC strain employed as a challenge in the study cited above, at 24 days of life (Lin, et al., 2013).



Keynote Speakers

We found the multi-subunit vaccine (K88 and LT) highly protective, with no or very minimal weight loss among vaccinates subsequent to challenge, and no death loss (Figs. 4-5). In contrast piglets receiving a vaccine containing only fimbriae exhibited significant weight loss as a group, and about 40% death loss. The LT-only vaccine was poorly protective, if at all. However, the diarrheal disease in these pigs progressed at a slower rate than was the case when pigs remained unvaccinated. Weight loss among LT-vaccinates was numerically less, but not significantly different than that of controls. Death loss was 57% compared with 74% among controls.

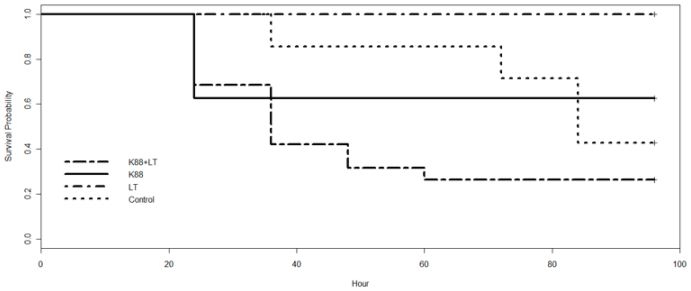


Fig. 5

Both studies suggest that the K88 fimbrial antigen is essential to an efficacious K88 ETEC vaccine and that LT also contributes to protection, perhaps either as an adjuvant enhancing immune responses to K88, or in stimulating antibodies to the toxin. Both studies also demonstrate that young piglets have immune systems sufficiently mature to mount protective immune responses quickly and in time to protect piglets from post-weaning ETEC disease, which may occur shortly after weaning. Neither study addresses whether vaccination will be successful when piglets are suckling their dams. However, since the subunit vaccine was delivered intranasally, and not intragastrically it seems unlikely that antibodies in the milk would block antigen binding and recognition in respiratory epithelium.

Returning to the study of a live vaccine, more recently we investigated whether its application could stimulate an immediate innate protective response. We reasoned that if this were the case, the vaccine could be delivered to piglets immediately after weaning, and in feed or water, such that handing of individual animals would be unnecessary. A product that could provide both short-term innate protection from disease and long-term immunity would not only be efficacious, but easily and inexpensively applied in a commercial setting.

Post-Challenge Observations Among K88-Receptor-Postivie Pigs Receiving Vaccine Strains 24h Before Challenge Table 3

Vaccine Strain Gene(s)	No. Pigs	Diarrhea	Weight change	Change in Serum Total Protein
K88	8	5	-2.9±0.1	-5.5±2.9
K88/LT(R192G)	7	0	-2.1±0.1	-3.3±4.3
K88/STb-LT(R192G)	7	0	0.1±0.1	-5.5±3.8
Non-Vaccinates	9	9	-19.3±0.1	30.6±6.3

In this study, we used an O8:H4 field isolate that expressed K88 fimbriae, but not LT or STb. The strain was transformed with a constructed plasmid containing a mutated LT gene, or a mutated LT gene fused with the STb gene. The mutation rendered the strain non-pathogenic in our hands. An empty plasmid (absent toxoid genes) was employed in the wild-type strain as a control of the toxoid genes. Piglets in this study were naturally farrowed from sows derived from a herd with animals known to possess the K88 receptor gene marker, and allowed to suckle 12 hours before separation from their dams, then were fed milk replacer for the duration of the study. At 5 days of age, piglets were inoculated orally with either vaccine strain, the empty plasmid control strain, or left uninoculated. Then, 24h later, all piglets were challenged with the same virulent ETEC strain utilized in previous challenge studies. All piglets were tested retrospectively for presence of K88 receptors and those without receptors were eliminated from data analysis. No piglets inoculated with either vaccine strain developed diarrhea and as groups, there was either no weight loss or weight loss of about 2% (Table 3). Piglets inoculated with the empty plasmid control strain were less protected, with 5 of 8 developing diarrhea, and a weight loss average of nearly 3%. All control pigs developed diarrhea, and weight loss on average was over 19%. From this study, it becomes clear that oral inoculation of young piglets with a living ETEC vaccine provides almost immediate protection from clinical disease. Thus, such a vaccine could feasibly be administered after pigs are weaned with the expectation of short term protection, presumably by stimulating a non-antibody based innate resistance, as well as long term immunity as addressed in our earlier study.

Alternatives to vaccine-based protection of pigs from ETEC.

In certain situations, such as in the face of a severe ETEC disease outbreak, an alternative to a vaccine-based approach to protecting pigs may be required. Polyphenols have been shown to have a positive regulatory effect on intestinal epithelium tight junctions (Suzuki, 2013). In addition, our colleagues and we have shown that certain polyphenolic compounds are highly effective in binding LT and other AB5 toxins. Therefore their use in pigs may provide some prophylactic or therapeutic benefit against ETEC. When we inoculated purified LT or cholera toxin into ligated intestinal loops prepared in young pigs, along with polyphenols from either grape skins or seeds, toxin-induced fluid accumulation was significantly blocked (Reddy et al., 2013). While the use of purified phenols in therapeutic applications for pigs likely would not be economically feasible, perhaps a similar protective effect might be had utilizing waste products from the wine industry, which includes grape seeds and skins. Such material could be lyophilized, milled and incorporated into the piglet diet.

Conclusions.

Enterotoxigenic E. coli causes diarrhea by first attaching to intestinal epithelial cells and there elaborating enterotoxins which precipitate fluid secretion. Most epithelial cells are initially refractory to bacterial attachment, suggesting that the bacterial receptor is initially held cryptic by the host. However, by a mechanism yet to be determined, it appears that bacterial adhesion to permissive enterocytes facilitates exposure of receptors of adjacent cells, allowing spread of colonization, which occurs in a mosaic pattern.

Piglets have sufficiently mature immune systems that if they are vaccinated by a mucosal route, they can be fully protected from ETEC disease by the time of weaning. Live constructed vaccines can be delivered by the oral route while subunit vaccines can be administered intranasally. In either case, efficacious K88 ETEC vaccines require antigens of both fimbriae and heat labile enterotoxin. A live constructed vaccine provides almost immediate protection against ETEC disease, possibly through up-regulation of non-antibody based resistance. Polyphenols if administered orally, may offer protection against the diarrheogenic effects of LT.

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Keynote Speakers

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Diagnostics for parasites - what do we know, where do we go?

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Ascaris suum is currently the only parasitic worm that is still highly prevalent in modern intensive pig production systems. However, due to the subclinical nature of the disease, ascariasis often remains undiagnosed, creating a lack of information regarding the worm-status of a farm, which ultimately makes it difficult for the farmers and the veterinarians to evaluate the applied deworming programs. In recent years, our group has developed and evaluated a serological test that can be used to measure parasitic exposure of fattening pigs more accurately. The test is based on the recognition of a haemoglobin protein (AsHb) produced by the parasite leading to a systemic IgG antibody response in exposed animals (Vlaminck et al., 2012). Evaluation of the test under field conditions have demonstrated a superior sensitivity for the detection of A. suum infections in comparison to faecal egg counts and liver white spots. A recent seroprevalence study performed with this test on over 500 European porc production facilities indicated the high prevalence of A. suum in fatteners, with approximately 50 % of the farms analysed testing positive. Furthermore, in order to increase our understanding of the impact of Ascaris on farm productivity, serological data of 20 fattening farms was compared with slaughterhouse data (such as percentage of affected livers, pleuritis and lung lesions) and farm performance parameters (such as feed conversion efficiency, days to market, daily weight gain, carcass quality and mortality). A significant correlation was observed between serology and production parameters, such as days to market and average daily growth, further indicating that A. suum infections can have a significant impact on farm economical parameters (Vlaminck et al., 2015). Furthermore, the results also indicate that serology forms an attractive new diagnostic tool that can be used to measure infection intensities. Currently, we are using serology to identify potential risk-factors associated with Ascaris infections and to measure the impact of applied control strategies. Also, the use of serology in sows and piglets is under further evaluation in order to determine the potential role of these animals in the widespread occurrence of Ascaris in fattening pigs.

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Keynote Speakers

From immunological understanding towards correct and new methods of vaccination in pigs

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Summary

In order to induce efficient protective immune responses to protect animals by vaccination against infectious diseases, it is imperative to have a good knowledge on the pathogenesis of the disease against which we want to vaccinate and to have a thorough understanding on the induction and effector fazes of the immune responses. In this presentation, we will focus on the antigen-processing and -presentation by antigen-presenting cells to T cells and their communication with B cells in order to generate effector and memory of cell-mediated and humoral antibody responses. In order to understand the required immune responses by vaccination, we will discuss the two general groups of vaccines, namely the replicating (live) and non-replicating (dead) vaccines, the implications of different routes of administration, their interference with possible already existing (passive or active) immune responses and the need and function of adjuvants. In the context of modulating the immune response towards inflammatory or less-inflammatory responses, we will also consider the activation of innate immune responses.

Introduction

For economic reasons, the pig industry is marked today by a phenotypic inbreeding for production parameters that might result in a genotypic linkage with an altered (compromised) immune responsiveness. A well-developed immune system and optimal immune responsiveness remain important for the welfare and productivity. Indeed these qualities can only be obtained if the health status of the animal scores high. Therefore a lot of energy and money is invested in prophylactic measures such as vaccinations and chemoprophylaxis against infectious diseases. However, health and immune responsiveness is not only maintained and improved by vaccinations and hygiene but also by an adequate supply of nutritional components to the animal.
In order to understand and improve prophylaxis by vaccination, it is imperative that we understand as much as possible the induction of an immune response by vaccination and the important communication (cell talk) between cells, such as antigen-presentation and cytokine production. A good comprehension of what is happening at the induction site of an immune response will help us to understand the difference between the use of live or dead vaccines as well as the interference of active (from a previous infection or vaccination) or passive immunity on the vaccination.

The induction of an immune response

1. The immune orchestra

An immune response is like music, produced by an orchestra (the immune-competent cells) under the guidance of a conductor (the professional antigen-presenting cell such as the interdigitating dendritic cell, IDC) who interprets the score (antigen) (figure 1). The immune responses can be divided in innate immunity, responsible for the first line of defense and induction of immune responses, and acquired immunity, responsible for antigen-specific memory. Innate immunity is mediated by the "so-called" antigen a-specific immune cells such as the IDC (the conductor), macrophages and neutrophils (microphages). However, their important immune role is not restricted to a first-line defense but lays also in the triggering of alarm signals (interpretation of the score and direction of the music) which are crucial for initiating and directing subsequent antigen a-specific and specific defense reactions (the music). Moreover, recent data have shown that these "so-called" a-specific cells are not so a-specific as they recognize and differentiate specific pathogen-associated molecular patterns (PAMPs) with pathogen recognition receptors, such as Toll-like receptors (TLRs). Depending on the PAMPs recognized, the APC can direct the immune response into opposite ways with important implications on the inflammatory response, production and protection (see later).

The antigen-specific cells (members of the orchestra) which have an immunological memory and secrete immuno-active molecules (the music), are divided in two main populations, the B and T cells (figure 1). Their antigen specificity is mediated by cell surface receptors (their instruments), the B cell receptor (membrane-bound immunoglobulin, BCR) and the T cell receptor (TCR), respectively. The repertoire of each of these receptors for their different antigens is clonally distributed, i.e. each individual T cell and B cell has only one idiotype set of antigen receptors and can thus only recognize one specific determinant or epitope of the antigen. BCR recognizes directly a specific natural epitope present on unprocessed antigen while TCR recognize epitopes of a processed antigen presented on MHC molecules of an antigen-presenting cell (conductor). Indeed, the antigen is processed intracellular (interpretation of the score) by an antigen-presenting cell (APC) into short peptides: linear epitopes of 8 to 20 amino acids long) that associate intracellular with major histocompatibility complex (MHC) molecules. These MHC molecules will, on subsequent expression on the cell membrane of the antigen-presenting cell, present the epitope to the T cell.

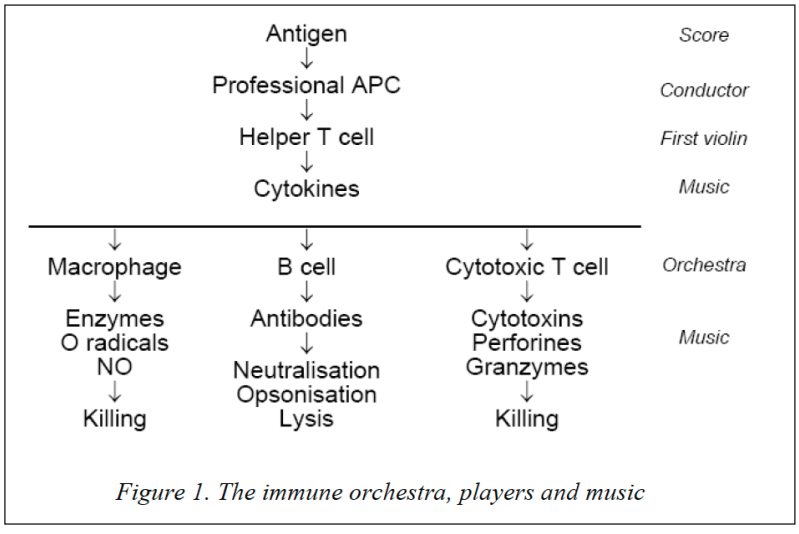

Figure 1. The immune orchestra, players and music

The T cell population is divided in CD4+ and CD8+ T cells (in swine there exist also double positive and double negative T cells) depending on the class of antigen-presenting MHC molecule they recognise. CD4+ T cells recognize their epitope in association with MHC class II molecules while CD8+ T cells recognize their epitope in association with MHC class I molecules. MHC class II molecules present epitopes from exogenous antigens, i.e. phagosomal antigens after uptake and processing by the APC (IDC, macrophage or B cell), while MHC class I molecules present epitopes from endogenous antigens, i.e. proteasomal antigens which have been transcribed inside the antigen-presenting cell as is the case for any virus-infected cell (figure 2). This difference has very important consequences as CD4+ T cells (or T helper cells, Th) compensate their antigenpresenting target cells with beneficial cytokines, while CD8+ T cells kill their infected target cells with cytotoxic factors and signal transductions.

Keynote Speakers

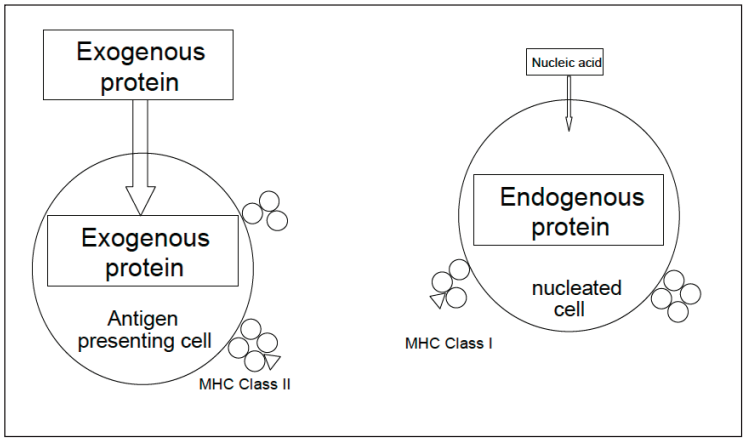

Figure 2. Presentation of processed antigens (peptides: T cell epitopes) of exogenous and endogenous antigens in association with MHC class II and MHC class I molecules, respectively.

Recently however, it has been demonstrated that in dendritic cells soluble antigens can be redirected during phagocytosis towards MHC class I presentation which has been named “cross-presentation”. For this cross-presentation, there appears to be a delay in the maturation of endosomes towards lysosomes under the control of NADPH oxidase-associated reactive oxygen radicals and endosomal antigen release by lipid peroxidation, enabling presentation by class I. Based on their cytokine-producing profiles, CD4+ T cells can be subdivided in different subtypes: the inflammatory T helper cell or Th1 is characterized by the secretion of interleukin-2 (IL-2), interferon- (IFN-) and IL-12 while the non-inflammatory T helper cell or Th2 is characterized by IL-4, IL-5, IL-10 and IL-13. More recently other T helper cells have been identified such as the antibacterial Th17 responsible for the attraction of neutrophils and the regulatory T cell (Treg) responsible for the production of immune-suppressive cytokines such as transforming growth factor (TGF-). The kind of CD4+ T cell that will be induced, is determined by the APC (IDC the conductor), the strength of stimulus and amount of antigen, and the kind of cytokine(s) produced by the environmental cells, all of which will have important modulatory implications on the type of resultant immune responses (music).

2. The concert hall: secondary lymphoid structures, the place of induction of B and T cells

Primary induction of immune responses occurs in the secondary lymphoid organs. These structures are filters for antigen (or antigen bearing APC) and naive lymphocytes and are optimally organized for intercellular communication between antigen-presenting cells, T cells and B cells. These organs are strategically placed on the lymph (lymph nodes) and blood circulation (spleen) to capture antigen and antigen-presenting cells draining from the tissues. By specific adhesion molecules, naive lymphocytes are directed to emigrate from the blood circulation into these secondary lymphoid organs in search for their specific antigen.

Conversely to other mammals, the draining lymph nodes of pigs appear rather an agglomerate of a few lymph nodes which are stuck together in an inverted position with the medulla being external to the cortex. Nevertheless, the physiology of the T and B cell areas are broadly conventional, with the T cell zone located in the paracortex and the B cell zone with its follicles located in the cortex. T and B cells enter via paracortical postcapillary venules, of which many are high endothelial venules (HEV). The major difference between pigs and other mammals lies in the emigration route of the lymphocytes: instead of leaving by sinuses through the medulla in the afferent lymph, they emigrate directly into the blood through the HEV. As a consequence, efferent lymph of pigs contains very few lymphoid cells as compared to other species.

The porcine spleen is composed of white and red pulp. The white pulp contains mainly leukocytes while the red pulp consists of erythrocytes and lymphocytes. In the white pulp lymphocytes are organised around the arterioles in peri-arteriolar lymphoid sheets (PALS). The peri-arteriolar sheet consists of a mantel of T cells (CD4+>CD8+) surrounded by a marginal zone containing macrophages and B cells. T cells are also found in the red pulp (CD8+>CD4+). These cells are already present at birth, but a large increase occurs after 2 weeks of life. At the same time, a similar increase occurs in B cells, mainly IgM+, with the formation of small follicles in the PALS as a consequence. In 4-week-old pigs the percentage of B cells in regard to the total amount of lymphoid cells, is similar to that in peripheral blood, approximately 35%. Most IgM-producing cells are located in the red pulp, whereas IgG- and IgA-producing cells are predominantly present in the periphery of the PALS. At 10 months of age however, IgG and IgA-producing cells can also be found in the red pulp.

3. Immunological defence of the gut and the gut-associated lymphoid tissue (GALT)

The immune defence system of the gut consists of lymphoid tissues and cells distributed along the gastrointestinal tract (reviewed in Brandtzaeg and Pabst, 2004). The lymphoid tissue localized along the gastrointestinal tract constitutes quantitatively the major part of the immune system of the whole body. This extremely developed gastrointestinal immune system reflects the importance of the mucosal immune defence system against the continuous attack of antigens and pathogens. Moreover, the major development of this local immune tissue as well as the individual immune reactivity seems to be induced by the continuous contact of the gastro-intestinal mucosa with the gastrointestinal flora, as well with pathogens, as evidenced by the atrophic mucosal immune system in axenic (germ-free) animals.

Some important features characterize the mucosal immune system:

- it possesses mucosa-associated lymphoid tissue (MALT or GALT for the gut-associated lymphoid tissue) as well as local and regional draining lymph nodes where the induction of immune responses is established, such as the Peyer's patches (PP) and the mesenterial lymph nodes, respectively,
- certain subpopulations of lymphoid cells predominate at the mucosal surfaces,
- there is a specific recirculation of mucosal lymphocytes towards mucosae, known as mucosal homing, and
- the predominant mucosal immunoglobulin is dimeric IgA which is secreted at the mucosal surface.

All these elements of the mucosal immune system are working together to generate immune responses that protect the host against mucosal invaders but also render the host tolerant against ubiquitous dietary antigens and the beneficial microbial flora of the mucosae. The elucidation of the mechanisms that determine immune tolerance or immune induction forms today a major topic of study where the type of activation state of the antigen-presenting IDC will determine whether T cells will or will not differentiate in T regulatory cells playing a role in immunotolerance .

The gastrointestinal immune system consists of lymphoid cells in organised sites like PP and mesenterial lymph nodes, and lymphocytes spread over the stromal tissues in the lamina propria and the epithelium (the intra-epithelial lymphocytes, IEL) of the intestine. There exists evidence that these organized structures are the places (or one of the places) where antigen enters the mucosal immune system to initiate subsequently the immune reactions: therefore these places are quite often called the inductive lymphoid sites of the mucosal immune system. Lymphocytes located in the lamina propria and between the epithelial cells of the intestinal tract are attributed with effector functions, such as antibody production, cytokine production and cytotoxicity, and these places are therefore referred to as the effector sites of the intestinal tract. These inductive and effector sites are interconnected by selective migration of lymphocytes (homing) whereby cells that have been activated in the inductive sites migrate specifically to effector sites of the intestinal tract. This interconnection assures that mucosal responses are primarily directed and localised against antigens that have been recognized at mucosal surfaces.

4. The overture: the acute phase response and the arachidonic acid cycle

Inflammation is the answer of tissue to irritation, injury and infection, and is quite often needed for the induction of strong immune responses. It consists of a complex cascade of nonspecific events, known as the acute-phase response, which confer early protection by limiting tissue injury to the place of infection or destruction. One important function of this reaction is to recruit more phagocytic cells to the site of injury. Moreover, it initiates the specific immune response against the invader. The localized reaction is induced by clotting factors and pro-inflammatory cytokines released by the activated resident sentinels, the tissue macrophages. The combined actions of their pro-inflammatory cytokines IL-1, IL-6, IL-12 and TNF- and the release of chemokines by activated macrophages and activated structural tissue cells (keratinocytes, fibroblasts, endothelial and epithelial cells) are responsible for changes in the surrounding capillaries, inducing an influx of neutrophils, monocytes and effector lymphocytes into the site of inflammation. Indeed, there is an increased expression of inflammatory adhesion molecules on the endothelial cells, which trap the circulating leukocytes with subsequent diapedesis and migration towards the place of tissue injury in order to fulfill their duties. However, activated phagocytes release also other proteins with potent local effects, such as toxic radicals, peroxides, nitric oxide, plasminogen activator and enzymes. Phospholipase A2 cleaves the fatty acid arachidonic acid (C20:4n6) from the glycerol backbone of membrane-bound phospholipids. The liberated arachidonic acid can then undergo controlled oxidative metabolism to form a variety of eicosanoids (C20) with different physiological and immune effects.

Keynote Speakers

The cyclooxygenase pathway yields prostaglandins (PG), prostacyclin and thromboxanes while the lipoxygenase pathway the leukotrienes (LT) and lipoxins. The eicosanoids act as autocrine/paracrine regulators, since most of their biological effects are limited to the site of biosynthesis. LTB4 stimulates chemotaxis of neutrophils and an increased expression of their C3b receptors while LTC4, LTD4 and LTE4 are collectively known as the slow reactive substance of anaphylaxis (SRS-A), being more than 1000 times more potent than histamine in smooth muscle contraction and vasodilatation. The latter is important in view of the increased blood flow in inflamed tissues inducing an even higher influx of leukocytes into the inflammation site. After exerting their effect, eicosanoids are rapidly catabolized to inactive compounds in the liver and lungs.

Vaccination: induction of a specific protective immunological memory

1. Live versus dead (inactivated, non-proliferating) vaccine

The absolute difference between live and dead vaccines lays in the fact that the vaccine dose of a dead vaccine required for successful immunization was made outside the host (the lab), while it is made inside the host for live vaccines. In other words, if a live vaccine fails to proliferate inside the host and fail to produce enough antigen to reach its immune dose, no immune induction or vaccination will occur.
The immunologically relevant difference however, between dead and live vaccines lays in the fact that a dead vaccine delivers exogenous antigens while a live vaccine delivers exogenous as well as endogenous antigens (figure 3). Although both vaccines induce good B cell (antibody) protective responses, dead vaccines induce only CD4 T cells while live vaccines induce CD8 as well as CD4 T cells. In view of intracellular pathogens, dead vaccines exert their effect on the infected cell indirectly by secreted cytokines while live vaccines do that by secreted cytokines and direct cell-mediated CD8 killing of the infected cell.

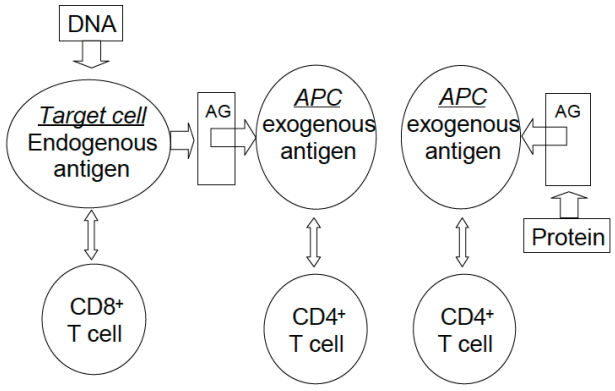

Figure 3. Presentation of processed exogenous and endogenous antigens to $CD4^+$ and $CD8^+$ T cells, respectively.

Another immunological relevant difference is that live vaccines can be administered systemically as well as mucosally while dead vaccines can for the moment only be administered systemically by local injection. As a live vaccine is delivered locally or mucosally, it will migrate to its predilection site and cells for proliferation, where it will induce a mucosal and systemic protective response. Conversely a dead vaccine will only induce a systemic response. However, major efforts in recent research are directed towards the mucosal delivery of dead vaccines or modulation of immune responses towards mucosae by systemic delivery of death vaccines. The last twenty years a lot of efforts have been directed towards oral delivery of antigens (in the feed) in order to immunize and protect animals mucosally at the gastrointestinal tract with dead (inactivated, non-proliferating) vaccines. The major challenge here is to avoid induction of antigen-specific tolerance which is a major mechanism operative against the multitude of antigens encountered at the gastrointestinal epithelium. One of the most promising ways of such oral antigen delivery for immunization is the receptor-mediated uptake at the gastro-intestinal tract which is and has thoroughly been researched, reviewed and discussed by our group (Devriendt et al. 2012). This mechanism is based on the uptake of molecules by receptors/ligands present on the intestinal epithelium (inclusive M cells of the PP) for lectins, immunoglobulins and even for some pathogen antigens and other molecules. The antigens of interest for immunization can then be recombinantly of covalently coupled to these molecules for receptor-mediated uptake in the body.
Such an optimised antigen uptake system opens ways for oral immunization via animal feed, where plants are then recombinantly transformed for production of the antigens of interest in leaves or seeds for inclusion in the feed. We tested already a recombinant system in plants for passive protection however, by specific expression of sIgA antibodies specific for F4 in seeds and inclusion of the latter in porcine feed with successful protection against weaning diarrhoea (Vikram et al. 2014).

2. Adjuvants

The generation of a successful immune response upon vaccination is dependent on several factors. We could summarize these factors in two categories, namely the antigen delivery, amount and presentation and secondly the creation of the correct immunological environment (inflammatory) or overture for inducing immune responses.

With respect to dead vaccines it imperative that the antigen is given over a prolonged period in the correct three-dimensional configuration (at least for correct and high affinity antibody formation) in order to have a correct and long-lasting antigen delivery and recognition. Therefore dead vaccines are delivered in precipitated forms or oil/water emulsions to avoid quick clearance from the body and to allow slow release of the antigen to the immune system. In order to get a good environment for immune induction, i.e. an inflammatory environment, inflammatory products are added in order to activate the innate immune system and antigen-presenting cells and increase the chemotaxis of immune-competent cells at the site of antigen delivery and immune induction (draining lymph node or spleen). Therefore adjuvants contain quite often ligands or pathogen-associated molecular patterns (PAMPs) which activate antigen-presenting cells as well as lymphocytes by binding to their pathogen recognition receptors such as Toll-like receptors, NOD receptors, RXR and c-type lectin receptors which belong to the innate immune system and converse with the acquired immunity to generate humoral and cell mediated immune responses.

In order to get maximal uptake of antigen by the antigen-presenting cells or the antigen-specific cells or to enable cross-linking of the antigen-specific receptors and as a consequence better activation of the latter, antigens are formulated in multimeric forms such as dendromers, iscoms, viroids, micells or liposomes.

A lot of efforts have also been focussed on the direction of immune responses of systemically delivered antigens towards the mucosae by selection and inclusion of specific adjuvants in immunization formulations (Cox et al. 2006).

With respect to live vaccines, the major point is that the vaccine is and stays alive before and upon administration allowing production of the required amounts of antigens for inducing the immune response inside the host and preferably at the entry and predilection site of the pathogen as the live vaccine (mostly attenuated pathogen) is migrating towards its preferred cells in that place. Antigens produced from live vaccines are normally in their correct tridimensional structure as well as in their multimeric form. When constructing live vaccines, immune-stimulating molecules can be introduced genetically by inserting coding genes or immune-stimulating nucleotide sequences (PAMPs) into the attenuated pathogen or live vector.

3. Maternal interference

One of the major problems in successful vaccination is the interference with maternal immunity or active immunity from previous vaccinations. Previous vaccinations or passive transfer of maternal antibodies via colostrum or milk can have a blocking effect on subsequent vaccination.

Indeed vaccination wants to induce or reactivate B cells and T cells via there membrane antigen-specific receptors, i.e. BCR and TCR respectively. When there are already secreted antigen-specific BCR i.e. antibodies (whether passive or active) circulating in the host, they can compete with the specific membrane BCR on B cells and as a consequence block the activation of the latter as the antigen epitopes are already opsonised by the circulating antibodies and cannot interact with the specific BCR for (re)activation of the B cells. As colostrogenic antibodies have been taking up in the systemic circulation while lactogenic antibodies remain in and protect the gastrointestinal tract, interference on systemic vaccination is mainly expected from colostrogenic antibodies.

Here again we have to make a difference between vaccination with live or dead vaccine. As the immunizing amount of antigen in dead vaccines has already been produced outside the host, circulating antibodies depending on their concentration, will only neutralise a certain part of the antigens. This can have an influence on the activation of B cells but less on T cells as they are reacting with T cell epitopes presented by antigen-processing cells and have not been neutralised by antibodies on B cell epitopes. On the contrary opsonised antigens might be favoured for uptake by antigen-presenting cells. Conversely, live vaccines have to produce their immunizing antigen amount inside the host and circulating antibodies can hinder or abolish that production by neutralising (killing) the live vaccine so that immune induction never happens, in other words it becomes a dead vaccine but containing much too low amounts of antigen to induce immune responses.

Keynote Speakers

Another way to avoid interference of systemic passive immunity on vaccination, is mucosal instead of systemic administration of the live vaccine. As the colostrogenic antibody level is much lower at mucosal sites than in the system, and as the mucosal site is quite often the predilection site for multiplication and transcription of the antigens, one would expect less interference by mucosal vaccination. This is probably the case for respiratory vaccination but not for oral administration as the gastrointestinal tract is full of lactogenic antibodies which might neutralise the vaccine.

Depending on the kind of passive immunity you want in the offspring, i.e. a high colostrogenic immunity or a high lactogenic immunity, vaccination time spots of the mother animal can be varied from distant to close to parturition date: 3 weeks prepartum will rather give a colostral immunity with high concentrations of IgG in the colostrum, while vaccination closer to partus will give longer and higher presence of IgG and IgA in the milk, enabling longer protection of gastrointestinal tract.

4. Interference between simultaneous vaccinations

Simultaneous vaccinations against different pathogens with separate dead vaccines might interfere with each other when injected in the same place, indeed when the injected vaccines are drained to the same draining lymph node where the immune induction is happening. This is all dependent on the kind of immune responses the vaccines are intended to induce. It is know that Th1 and Th2 responses interfere with each other in the kind of immune response they induce by their secretion of opposing cytokines. As some adjuvants are added to direct immune responses towards rather humoral or cell-mediated immune responses, they can have opposing effects on their respective immune inductions. As these different factors present in the vaccines or produced during vaccination might interfere with each other, it is advisable to test first in vivo if they are or are not interfering with each other in the induction of their respective intended immune responses.

When the vaccines are however injected in different places drained by different lymph nodes, no interference is expected. The only interference which might happen would be that one vaccine is so inflammatory that most immuno-competent naïve cells are drawn towards that vaccine-induced inflammation site so that there is a decreased blood flow in the other vaccination site with reduced chance or slower timing to find the correct antigen-specific cells to be stimulated.

Conversely, administration of a single vaccine containing antigens from different pathogens is safe and should not interfere in the induction of protective responses against the different pathogens as this has (should have been) been tested in the clinical trials by the company: the adjuvant for inducing the type of T cell response has been selected on the basis of what type of protective responses are desired against the respective pathogens. However, it is still possible that certain molecules of specific pathogens act as PAMP and might have their own effect of the direction of the immune response.

Conclusion

In order to understand and improve vaccine choice or vaccination schedule, it is imperative to comprehend the music of immunology. Live vaccines are immunologically speaking the best as they can be delivered at the entry port of the pathogen (i.e. mucosae) where the latter is best stopped to disseminate into the body and secondly as they induce cell-mediated, inclusive CD8 responses, as well as humoral immunity. However, vaccinations with live vaccines are more sensitive to failure than those with dead vaccines, as they can fail in reproduction and production of their necessary antigenic load for immune induction. In conclusion, vaccinate animals with live vaccines when, or in places where no interference is expected and vaccinate with dead vaccines when passive or active immunity is already present and might interfere.

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“Is control of LA-MRSA in pigs possible and relevant? – Experiences from Norway”

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Background

Staphylococcus aureus is an important cause of nosocomial and community-acquired human disease, and methicillin-resistant S. aureus (MRSA) is associated with increased morbidity, mortality and costs [1]. During the last decade, particular clones of MRSA called livestock-associated MRSA (LA-MRSA), due to their ability to colonize and persist in livestock, has emerged in most European countries [2]. The most widespread LA-MRSA in Europe and Northern-America belongs to the clonal complex (CC) 398, and has been reported particularly from pigs and veal calves [3]. S. aureus, particularly LA-MRSA, is not regarded an important cause of illness in pigs, and pigs are predominately either colonized or contaminated without any signs of infection.

Internationally, the emergence of LA-MRSA in livestock has raised public health concerns [4]. For instance, in Denmark, a pig-dense country with a low prevalence of MRSA in the human population, CC398 has within a few years become the most commonly detected CC group among humans [5]. Studies has shown less human-to-human transmission of LA-MRSA than other MRSA, and that persons occupationally exposed to pigs or veal calves, and to a lesser extent their family members, are most at risk of being positive for LA-MRSA [3, 6]. More recently, LA-MRSA has also been described in persons not occupationally exposed to pigs, but living in areas with a high pig density [5, 7].

Experiences from Norway

The Norwegian pig population consists of approx. 1250 sow farms and 800 finishing pig farms with an annual production of 1.6 million slaughtered pigs. Average herd size per 2014 was 118 sows/year including sow pool systems (n=13). The production chain is organized in a pyramid structure with pure breed nucleus herds (n=40) at the top and multiplier herds (n=55) supplying the commercial sow herds (approx. 1150).

The first detection of MRSA CC398 spa-type t034 occurred through an anonymized abattoir survey in 2011 finding MRSA in samples from a single slaughterhouse [8]. The following year, an anonymized pig holding survey demonstrated CC398 in samples from a single farm of the 175 investigated [9]. In addition to the EU Baseline study investigating 252 farms [10] that only detected one human associated MRSA, these results indicated a very low prevalence of LA-MRSA in the Norwegian pig population before 2013.

Public health concerns were the rationale behind the decision made by the Norwegian authorities in 2013 to impose measures to eradicate LA-MRSA from the pig population. This “search and destroy” strategy aims to prevent pig holdings becoming a persisting domestic reservoir of MRSA with the potential of zoonotic transmission. To the authors’ knowledge, Norway is the only country having implemented such a strategy. This strategy was implemented following two separate and traceable detections of CC398 in early 2013; in samples from a fattening pig submitted for post-mortem examination and a clinical isolate from a hospitalized farm worker, respectively. These findings led to two extensive outbreak investigations in the eastern and south western part of Norway, respectively, identifying a total of 24 pig farms positive for CC398.

The LA-MRSA eradication strategy includes restrictions on trade of live animals upon suspicion, depopulation of pigs in LA-MRSA positive pig holdings, thorough cleaning and disinfection of premises, a mandatory down-time and negative environmental samples from the pig barns before restocking with pigs from MRSA negative holdings. After restocking, samples are collected from animals and the environment several times to assess the effectiveness of MRSA eradication. The detailed plans and execution of the farm specific measures of cleaning and disinfection were the responsibility of the farm owner, often assisted by farm consultants from the Pig Health Service. Personnel in contact with positive pig

Keynote Speakers

holdings were sampled by local health care providers, and received treatment to eliminate carriage of MRSA if found positive. In Norway, MRSA in humans is a notifiable infection. Epidemiological information was collected through a purpose built questionnaire from all farms where MRSA was detected.

During 2014, all Norwegian sow farms with an inventory of more than 10 sows (n=986) were included in a national surveillance program for MRSA in pig holdings. From each farm, pooled swab cloths were collected from animals and the interior environment. This screening resulted in the finding of a single LA-MRSA positive sow farm [11]. In 2015, the surveillance program for MRSA in pigs included all nucleus and multiplier breeding farms and all fattening pig farms (n=821). In this screening, LA-MRSA was detected in four fattening pig farms and one multiplier breeding farm [12]. These two screening studies provide evidence of a continued low prevalence of LA-MRSA in the Norwegian pig population. The surveillance is continuing with a sow herd screening in 2016.

From the first traceable findings of MRSA CC398 in 2013 and until the end of December 2015, LA-MRSA has been detected in 6 separate outbreaks including a total of 60 herds in Norway. This includes all farms identified through active surveillance or outbreak investigations. In each outbreak, the primary introductions are suspected to be humans. This is in contrast to the 2010 EFSA report, identifying trade of live pigs as a major risk factor for transboundary spread of LA-MRSA [13]. Import of live pigs to Norway from other countries is negligible [14], and this is considered an important epidemiological and biosecurity feature of the Norwegian commercial pig population. However, the predominant route of further transmission within the country has been through the trade of live pigs. Whole genome sequencing was performed on MRSA isolates from all MRSA positive pig farms.

Results from follow-up testing after restocking are available from 32 pig farms that have performed MRSA eradication. In 29 (91%) of these farms, MRSA eradication was successful after the first attempt. One herd was closed after depopulation and has not been restocked. The remaining farms have not yet completed the eradication and/or follow-up sampling due to the short period of time that has passed since detection. Thereby, the conclusion is that MRSA eradication is possible in Norwegian pig farms.

Population surveillance, outbreak investigations and measure to eradicate LA-MRSA from pig farms is both a costly and labor intensive strategy. However, the imposed strategy has probably contributed substantially in preventing further dissemination of LA-MRSA, and in preventing an increased prevalence of LA-MRSA among pig farms and humans in Norway [15]. The strategy is therefore considered relevant under Norwegian conditions, presently characterized by; a low overall prevalence of MRSA (including LA-MRSA) in humans [15], few primary introductions of LA-MRSA to the pig population, effective eradication of MRSA from positive pig farms which thereby prevented further transmission among pig farms, and an essentially closed pig population. Any changes to any of these conditions may influence the authorities' choice of strategy regarding LA-MRSA in the future.

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Keynote Speakers

Do we need to vaccinate against parasites? If yes, which one? Are you sure?

Vaccination against parasites – status quo and the way forward

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Introduction - vaccines against parasites

In modern swine medicine, vaccination against various pathogens is an integral part of the health management. However, currently not a single vaccine against parasites of swine is commercially available. Compared to viral and bacterial pathogens, there is a general scarcity for anti-parasite vaccines; only two anti-nematode vaccines, one anti-tick-vaccine and a handful of antiprotozoal vaccines are available for domestic animals. The reasons for such a limited number of anti-parasite vaccines are manifold. In many parasites the development of immunity is slow, and especially in livestock animals, the required time is too short for the vaccine to be of value before animals go to slaughter.

Since parasites, especially helminths, are prime manipulators of the immune system, immunity is often also incomplete and not sufficient to interrupt the life cycle, which aids in the continuation of transmission in a population regardless of vaccination. For host species with a fast turnover the development of anti-parasite vaccines are considered too expensive and especially in intensive pig (or poultry) production chemical control is cheaper, easier to apply and is considered to have a broader market. The only anti-parasite vaccine currently available for use in pigs, the anti-Cysticercus cellulosae-vaccine for the prevention of porcine cysticercosis (which leads to infection of humans with the tapeworm Taenia solium), is not commercially available although it has a very good efficacy (Lightowlers, 2013). This parasite, although a major zoonotic agent on a global scale, is simply of insufficient economic importance for the pork industry to attract sponsors from pharmaceutical companies.

However, the development of vaccines against parasites is still a significant research topic in medical and veterinary sciences. Pathogenic and therefore economically important diseases, especially those which are insufficiently controlled by available chemotherapeutics or have developed resistance against them that cannot be immediately overcome, are still in the focus. In addition, zoonotic parasites represent an attractive target under the One Health aspect, and finally there is a growing public interest in organic production of food free of chemicals, which is fostered by consumer concern about drug residues in meat, eggs or milk.

Which swine parasites are to be considered for vaccines?

The only group of vaccines against parasites that is well developed are the anti-coccidial vaccines for poultry, i.e. Eimeria in chicken and turkey. Live virulent and attenuated vaccines are the predominant types on the market; some strains have been used for more than 50 year without significant alterations (Strube and Daugschies, 2015). Technically, vaccination with life parasites, in this case Eimeria oocysts from several relevant species, represents an infection of susceptible animals under controlled conditions. The parasites undergo the complete life cycle and recirculation of oocysts induces a natural booster, rendering chicken immune after several cycles of reproduction. It is assumed that vaccine strains which are susceptible to anticoccidials can displace resistant field isolates when applied repeatedly (Chapman and Jeffers, 2014).

In order to be a candidate for vaccine development, parasites have to fulfil several prerequisites. They must be sufficiently pathogenic to induce disease and or/economic losses that can be ameliorated by vaccination, and natural infections must be immunogenic and induce protective immunity and an immunological memory. Amongst the most common swine parasites, some fulfil these criteria. Sarcoptes scabiei var. suis, the mange mite of pigs, causes severe

economic losses and frequently serious disease in pigs when untreated (Davies, 1995). Currently, control of porcine sarcoptic mange relies on the application of acaricides and the maintenance of mite-free herds (Laha, 2015). Immunity against scabies has been described in different species including humans (Walton, 2010). Vaccination has been attempted in rodent models (Gu et al., 2014) and other species, and it might also be feasible in pigs. Strongyloides ransomi is a nematode which is most commonly transmitted with the colostrum after reactivation of hypobiotic larvae in the sow. It causes transient diarrhoea in suckling piglets and induces strong immunity in the adult intestinal stage which leads to rapid expulsion by the host. The immune mechanisms of expulsion have been investigated for other Strongyloides species (Yasuda et al., 2014), therefore this nematode also fulfils the principle criteria for a vaccination candidate. Currently, however, its control using broad-spectrum drugs such as macrocyclic lactones is considered sufficient and anthelmintic resistance has not been reported. This is also true for other nematode species of swine that are expelled by action of the gut immune system in pigs, the large roundworm, Ascaris suum (Masure et al., 2013), and the whipworm, Trichuris suis. In contrast to these the nodule worm Oesophagostomum induces only a weak reaction of the host's immune system (Andreasen et al., 2016), making the latter unsuitable for immunological intervention.
Of the protozoa, Toxoplasma gondii is an attractive candidate in vaccine development, as it is the most important foodborne zoonotic parasite on a global scale and interruption of the life cycle by preventing cyst formation in animals used for meat production would effectively truncate foodborne transmission. A range of promising vaccine designs and candidates has been used in mice (Lim and Othman, 2014), and also in pigs (e.g. Burrells et al., 2015 for recent works). While it is assumed that vaccination of livestock against Toxoplasma can prevent infection in humans, the infection in pigs causes only minor production losses or animal health problems and the attractiveness of such a vaccine for pig producers is certainly only limited unless the label "Toxoplasma-free pork" has economic advantages. In suckling piglets Cystoisospora suis (syn. Isospora suis) causes intestinal infections which may cause transient diarrhoea mostly in the second week of life. Due to the peculiarities of the porcine neonatal immune system (see below) and the strong age resistance to C. suis in piglets older than three weeks (Worliczek et al., 2009a, 2009b), it is currently assumed that only pigs older than six weeks can mount an appropriate immune response. For this and other reasons vaccination of piglets against C. suis is not considered feasible. However, alternative approaches have recently been evaluated (see below).

Parasite control in swine production – current status

Currently, antiparasitic treatment schemes for pigs is comprised of a "standard" application scheme for different production branches; they are not "tailor-made" or risk-based and not driven by diagnosis, since metaphylactic application of antiparasitic drugs during the prepatent phase (when parasites cannot readily be detected by routine screening) is preferred to prevent dissemination of environmental stages (especially nematode eggs or coccidia oocysts) and infection of the litter or herd. Complete elimination from a herd is often difficult to achieve due to high prevalences, frequent distribution and durable environmental stages, the best example being eggs of Ascaris suum which are almost impossible to inactivate and which can remain infectious for years under suitable conditions (Wiwanitkit and Wiwanitkit, 2013). An exception is the control of mange; S. scabiei has no long-lived environmental stage and relies on direct contact for transmission, so systematic application of acaricides can effectively reduce infection and stamping out the parasite on a farm is possible when quarantine measures and proper diagnostic screening are in place (e.g. Smets et al., 1999). However, sustainability is jeopardized by the development of acaricide resistance as reported from human scabies (Lopatina, 2012). Integrated measures like complete all-in-all-out and disinfection with effective chemicals can relieve the infection pressure of endoparasite infections (Joachim et al., 2001) but eradication is generally considered not feasible. Although resistance against anthelmintics in pig nematodes seems to be restricted to Oesophagostomum at low frequencies (Várady et al., 1996; Bauer and Gerwert, 2002; Gerwert et al., 2002) and resistance to anticoccidials in the control of C. suis is currently not reported, the limited number of substances available especially for parasite control in pigs is of concern; especially because no routine tests are available for the detection of parasiticide resistance, and no programs to delay the development of resistance (like shuttle programs as for chicken coccidiosis prevention or equine cyathostominosis) are in place. Alternative control strategies (for review see Roepstorff et al., 2011) haven been shown to be effective but are currently not commercially available. It must therefore be assumed that, although currently parasites may not be considered as an issue of major concern in pig health and production, in the long run alternatives to the current chemotherapy must be sought to maintain appropriate control and efficacy of available drugs.

Keynote Speakers

Consequences of neonatal enteric infections: parasites and their buddies

At the time of birth the porcine immune system is poorly developed (for review see Worliczek et al., 2009b); intestinal Peyer's patches contain almost no immune cells and the gut epithelium and subepithelial tissues are only completely populated with T- and B-cells and antigen-presenting cells at about six weeks of age, leaving ample time for pathogens to establish and reproduce. At the same time, the gut microbiota are establishing and infections with pathogens at a very early age may have a number of consequences beyond transient parasite infection. Synergistic effects have been described for C. suis and toxigenic Clostridium perfringens where timely anticoccidial treatment also alleviated the effects of clostridiosis (Mengel et al., 2012), showing that C. suis infections promote adhesion of clostridia to the intestinal mucosa, exacerbating the effects of bacterial infection. Preliminary studies also indicated that infections with C. suis alter the succession of bacterial communities in neonatal pig gut, delaying the establishment of lactobacilli (as reviewed in Shrestha et al., 2015). As interactions between microbiota and the immune system are key to in the development of a functional immune system (Swiatczak and Cohen, 2015) such events may have lasting effects on the development of intestinal and immune functions. As such alterations have been described for other intestinal parasitic infections (Loke and Lim, 2015), a role of intestinal parasitic infections in gut health should be re-evaluated in pigs, too.

Cystoisospora suis - a candidate for vaccination?

As mentioned above, C. suis is an important cause of neonatal diarrhoea; infected animals excrete several million oocysts in the patent phase of infection and the dissemination within and between litters accounts for a rapid spread of the parasite with the consequence of transmission to the majority of piglets within the first week after birth. Although infections are transient with creamy to watery non-haemorrhagic diarrhoea for one to six days, affected animals often develop poorly and stay smaller even until weaning compared to healthy (treated) litter mates (McOrist et al., 2010), which accounts for the financial losses attributed to this disease (Scala et al., 2009; Kreiner et al., 2011) requiring treatment. In addition, dysbiosis may contribute to increased morbidity (see above) and require antibiotic treatment (Driesen et al., 1995). Good control of oocyst excretion and coccidiosis-related diarrhoea is achieved by metaphylactic treatment of piglets on the third to fifth day of life with a single dose of toltrazuril (20 mg/kg of body weight) but recently questions about the sustainability of "blanket treatment" of piglets in terms of resistance and drug residues in meat have risen (see Shrestha et al., 2015), and a call for alterative control strategies has been voiced.

C. suis as a member of the Apicomplexa, which have a strictly intracellular development in the host, was assumed to be under the control of the cellular immune system, mainly NK cells, CD4+ and CD8+ T-cells, while antibodies are probably not protective (Worliczek et al., 2009b). Phenotyping of cells after primary infection revealed that several subpopulations of T-cells (especially -T cells and, TH cells) were deceased in peripheral organs (blood, spleen) but increased in the jejunum upon infection), and after challenge infection (5 months after primary infection) these cell populations also produced interferon- (which is crucial for the defence against apicomplexan parasites) and were able to proliferate upon antigen stimulation (Worliczek et al., 2010a, 2010b; Gabner et al., 2014). Thus, despite considerable individual reactions, it can be assumed that C. suis can induce specific primary and adaptive immune reactions in pigs including the induction of an immunological memory.

Since a relative of C. suis, Cryptosporidium parvum which also inhabits the epithelium of the small intestine, is at least partially controlled by specific antibodies (Martín-Gómez et al., 2005), investigations in the possible role of anti-C. suis antibodies were made and colostral transfer of antibodies resulting in high serum levels in piglets was described, and IgA levels in the blood of piglets experimentally infected with C. suis soon after birth were negatively correlated with diarrhoea (Schwarz et al., 2013). When sows were inoculated before birth with high doses of C. suis oocysts, no clinical signs or oocyst excretion were noticed but the levels of immunoglobulins (especially IgA) in their blood, colostrum and milk were correlated with a decrease in diarrhoea and oocyst excretion in their experimentally infected off spring compared to piglets from non-superinfected sows (Schwarz et al., 2014), indicating that application of oocysts to sows ante partum can confer at least partial protection against C. suis in piglets. It was also concluded that the role of the sow in spreading the parasite is probably minor since even after infection with high doses (100,000 oocysts / sow) no shedding was observed; however in the immunity against C. suis the role of the mother in the provision of colostrum containing protective substances is probably pivotal. From this it is currently assumed that immunological control of

neonatal porcine cystoisosporosis would have to be conferred as a maternal vaccination which could be applied in time before the infection of the newborn piglets to immunologically mature gilts or sows and could be boostered by natural infections circulating in a herd.

Determination of vaccine candidates – the way forward

Current vaccines against coccidian in chicken are mostly virulent or attenuated live vaccines (see above). This technology requires the use of animals for production of vaccines with all its disadvantages (biosafety, ethical concerns etc.). Lately, a subunit vaccine against Eimeria maxima of chicken was developed and marketed for use in maternal immunization (Wallach et al., 2008; Sharmann et al., 2010). Although it is also produced in animals and its lasting success in the field still remains to be evaluated, the concept of inactivated vaccines against parasites has received a significant incentive with this development. Until recently, the search for new vaccine candidate molecules was slow and cumbersome due to the lack of cost-effective high yield / high throughput in vitro techniques for parasite propagation and screening. New techniques in biotechnology and bioinformatics have enabled rapid and cost-effective screening of genomes and transcriptomes of parasites for vaccine and drug target candidates (as reviewed in Cantacessi et al., 2012; Jex et al., 2013, and others) including A. suum (Jex et al., 2011), T. suis (Jex et al., 2014), T. gondii (www.toxodb.org) and C. suis (Palmieri, unpublished). For C. suis a genome size of 83 Megabases encoding >8,300 genes is estimated, and a recently developed pipeline for the search of vaccine candidates in apicomplexan parasites (Vacceed; Goodswen et al., 2014) has detected 562 candidates in C. suis which now need to be evaluated further in silico, in vitro (using a cell culture system supporting the complete life cycle of C. suis; Worliczek et al, 2013) and in vivo.

Conclusion

In summary, although vaccines against porcine parasites do not seem to be an immediate issue for pig industry and health, the time to get started has never been better, as new tools and technologies are greatly accelerating the achievements in this field of veterinary medicine.
C. suis would be an attractive candidate for vaccine development, as preliminary data have shown that a protective effect could be achieved by immunisation of sows; however, more basic and applied research will be needed to fully understand the mechanisms that lead to protection.
Joint forces of the veterinary profession, immunology, bioinformatics and biotechnology specialists will be required to develop and test new vaccine concepts and vaccines to be suitable for a competitive market. Combinations of vaccines and optimised delivery systems will have to be developed alongside to incorporate anti-parasite vaccines into the health management systems of pig production.

The author declares that there are no conflicts of interest.

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Keynote Speakers

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Factors for high reproductive performance of sows in commercial herds

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Introduction

With information technology, commercial herds have collected and stored many data. New technologies are expanding the possibilities for data collection, information-exchange, collaboration and data analysis. However, the use of these data have been limited. Farm data analysis can help veterinarians and producers to identify a production problem that they did not recognize and make a better decision about solutions. Furthermore, data analysis could increase the dissemination of useful information to improve herd productivity and stable outputs in breeding herds. It could identify important factors associated with reproductive performance in order to maximize sows' reproductive potential. In this paper, we review factors associated with sow performance and herd productivity in commercial herds. The factors include both sow level factors and herd level factors. With regard to sow level factors, there are risk factors and other factors. Reproductive performance is not a disease and there is a case that the risk factor is not an appropriate term. For example, PBA is not a risk factor, but actually a predicting factor for lifetime prolificacy of sows (Iida et al., 2015). Factors at the herd level include high-performing herds, herd management factors and herd size. Additionally, boar factors should also be considered in order to improve reproductive performance of sows.

A. Forty pigs weaned per sow per year

The number of pigs weaned per sow per year (PWSY) is commonly used as a benchmarking measurement to compare the productivity of breeding herds between herds or countries. The target values for PWSY have been increased from 20 to 30 pigs over the last three decades, and it is likely that 40 PWSY will be the next target in the swine industry. To achieve 40 PWSY, it is necessary to obtain both 17.3 pigs weaned per sow and 2.3 litters per sow per year by assuming 28 days of lactation, 115 days of gestation and 36 days of non-productive sow days (Dial et al., 1992; Almond et al., 2006). It is likely that genetics and sow management can increase PWSY up to 40 pigs in the near future. However, even though PWSY is a good measurement for herd productivity in the short term, it is not the best measurement for the longevity nor the welfare of piglets or sows. There is a concern that herds with 40 PWSY may produce many runts or small piglets. So welfare in piglets may be compromised when we genetically increase sow prolificacy to such a high level, unless genetic improvement is directed to increasing the uterine capacity, number of functional teats and milk production in sows.

B. Reproductive performance in commercial herds

Sow reproductive performance

In the productivity tree of breeding herds (Dial et al., 1992), there are two branches: one is the number of pigs weaned per sow, and the other is the number of litters per sow per year. The number of pigs weaned depends on the number of pigs born alive and preweaning mortality; the number of litters per sow per year depends on non-productive days, lactation length and gestation length.

Sow reproductive performance includes both fertility (e.g. farrowing rate: FR and weaning-to-mating interval: WMI) and prolificacy (e.g. pigs born alive: PBA). In terms of fertility, the number of litters per sow per year is affected by FR and WMI as well as reservice interval and culling interval, via their effects on non-productive days. Meanwhile, prolificacy is determined by the effects of PBA and preweaning mortality on the number of pigs weaned.

Sow mortality is related to fertility because increased mortality increases death intervals and non-productive days which decreases lifetime fertility in sows. Also, abortion occurrences in commercial herds increase non-productive days of gilts and sows (Iida et al., 2016).

Lifetime performance

Mean sow life days is approximately 1,000 days in southern European countries and Japan. It is important for producers to maximize reproductive potential during sows' lifetime in order to decrease production costs and economic inefficiency in commercial breeding herds (Stalder et al., 2012).

Lifetime performance includes longevity, that is measured as the number of parity or age at culling or removal, and also lifetime PBA, lifetime number of pigs weaned and lifetime non-productive sow days (Sasaki et al., 2011). Annualized lifetime PBA is an integrated prolificacy measurement for sows that combines lifetime PBA with lifetime non-productive sow days. In contrast, annualized lifetime pigs weaned can be considered as an integrated measurement of sows' lifetime reproductive productivity that combines sow performance (i.e. PBA and preweaning mortality) with lactation management including nursing and fostering techniques. For example, annualized lifetime pigs born alive per sow is calculated as the number of lifetime pigs born alive divided by the sow's reproductive herd life days x 365 days. The sow's reproductive herd life days is the number of days from the date that the sow was first-mated to its removal. For replacement gilts, the date of first-mating appears to be more appropriate than herd entry date, because the herd entry date varies between herds.

C. Sow level information- factors for sow performance

Low or high parity

Low parity females, especially pregnant gilts and parity 1 sows, have lower reproductive performance than parities 2-5 sows, such as lower FR, higher returns, lower PBA and higher WMI. As the number of parity increases, reproductive performance also increases, reaching a peak between parities 2-5 before it then declines. For example, PBA is highest between parities 3 and 5, whereas FR is highest between parities 2 and 4. Parity 1 sows also have a prolonged WMI which can be explained by the immature endocrine system in these growing young animals, and their low feed intake during lactation which decreases LH secretion (Koketsu et al., 1996a) leading to restricted follicle growth in their ovaries. Parity 1 sows in commercial breeding herds may not consume sufficient nutrients and energy to grow and reach their mature reproductive performance level.

Aged sows also have lower reproductive performance than parities 2-5 sows. There are various reasons for the lower performance. For example, ovulation and fertilization rates decrease in aged sows. Also, their embryonic mortality or pregnancy loss and the number of stillborn piglets increase due to slow responses to the space demands by growing fetuses and to the stimuli from parturition processes (Almond et al., 2006). Additionally, aged sows (parity 5 or higher sows), and also gilts, are at higher risk of having abortion than parities 3-5 sows (Iida et al., 2016).

Season or climate factors

Fertility and prolificacy measurements decrease during summer months. For example, FR is lowest in summer, and also PBA in summer-mated sows is lower than for winter or spring mated-sows. It has been hypothesized that the summer reduction in reproductive performance occurs through a combination of high summer temperatures reducing GnRH secretion, and impaired ovarian follicle development that leads to compromised corpus lutea functions secreting low progesterone concentrations (Bertoldo et al., 2012).

Various studies have highlighted important climatic factors related to the seasonal effects, including daily maximum and minimum temperature, humidity and photoperiod. Climate data in Meteorological stations near studied herds have been used to quantify the association between high temperatures and sow performance (Tummaruk, 2012; Bloemhof et al., 2013; Iida and Koketsu, 2013; 2014b). For example, increased outside temperature decreases FR and total number of pigs born, while it increases returns, WMI and mortality. Also, the impact of the summer effect or outdoor temperature on reproductive performance varies depending on parity number. A previous study showed that as outside temperature increased from 25 to 30 C0, the total number of pigs born to parity 1 decreases by 0.6 pigs at their subsequent parity, whereas for parity 0 females the decreases at subsequent parity was only 0.2 pigs (Iida and Koketsu, 2014b). Another example is that WMI in parity 1 sows increased by 0.8 days as daily maximum temperature rose from 25 to 35 °C,

Keynote Speakers

whereas in parities 2 or higher sows the increase in WMI was only 0.3 days (Iida and Koketsu, 2013). These results indicate that that parity 1 sows are more sensitive to such summer changes in climate than gilts or sows at parity 2 or higher. This type of sensitivity appears to be related to the immature endocrine system in parity 1 sows and the low feed intake of parity 1 sows during lactation.

Lactation feed intake and its patterns

Lower lactation feed intake is associated with lower average weaning weight, prolonged WMI, low FR, more returns or more culled sows due to reproductive failure, and fewer PBA at subsequent parity (Koketsu et al., 1996b). This is particularly the case with parity 1 sows where low feed intake during lactation is a detrimental factor related to post weaning reproductive performance such as WMI and FR. Some lactational feed intake patterns (e.g., major dip) are related to prolonged WMI and more culled sows due to reproductive failure. However, increased lactation length and advanced automatic feeders for lactating sows may change these risks to reproductive performance.

Lactation length

There has been a concern about early weaning systems in the U.S.A. being associated with suboptimal reproductive performance, such as low FR, prolonged WMI and fewer PBA at subsequent parity (Koketsu et al., 1998). Also, short lactation length decreases average feed intake during lactation. However, since 2000, the U.S.A. swine industry has been moving from early weaning to increased lactation length (Knauer and Hostetler, 2013) to increase improve growth performance in nursery and grower pigs. Also, in the European Union the weaning of piglets from the sow at less than 28 days of age has been prohibited since 2013 (European commission: Animal welfare practices, 2015). Meanwhile, there is another concern that some nurse sows with increased lactation length lose their body reserve much due to high milk yields, so they may have increased prolonged WMI and lower FR.

Number of inseminations or matings

Deciding the ideal number of inseminations is related to optimizing reproductive performance and minimizing costs. Single insemination, due in part to late timing, is related to low FR (Kaneko et al., 2013). Inseminating two times with accurate heat detection is more cost-effective than three times inseminations in terms of the costs for labor, semen and a catheter (Takai et al., 2010). However, a GnRH antagonist given intravaginally in gel form has been shown to be effective at advancing and synchronizing ovulation (Knox et al., 2014). Using this GnRH technology, a single insemination has been practiced in the U.S.A. industry enabling reduced costs while still having reproductive performance similar with multiple inseminations.

Peri-partum period or farrowing event

Sow mortality is an indicator of maternal health and animal welfare. Farrowing is a major risk factor for sows in all parities and seasons. A previous study showed approximately 68% sow deaths occurred in the period from 4 weeks before farrowing to 4 weeks after farrowing (Iida and Koketsu, 2014a). As the number of parity increases, the mortality risk for sows also increases. So aged sows in high parity (e.g., parity 6 or higher) in the peri-partum period are at the highest risk of dying (Sasaki and Koketsu, 2008).

It has also been shown that in subtropical climate zones mortality increases in low parity sows increases during summer, whereas in aged sows it increases during winter (Iida and Koketsu, 2014a). It appears that lower parity females that have immature bodies are more sensitive to high ambient temperature than multiparous sows. Pigs are particularly susceptible to heat stress because they have a weak cardiovascular system and limited sweat glands (Frazer, 1970). Distortions in abdominal organs and heart failure are major causes for death in female pigs (Stalder et al., 2012). Similar cardiovascular problems can occur in (human) women; for example, peripartum cardiomyopathy is reported as a disorder in which initial left ventricular systolic dysfunction and heart failure occur (Silwa et al., 2006).

Aged sows are more sensitive to low temperature than low parity females in subtropical climate zones (Iida and Koketsu, 2014a). In humans also, the prevalence of gestational hypertension, pre-eclampsia and eclampsia are highest with delivery in the winter months (TePoel et al., 2011). Such diseases may be related to aged sow responses to cold or to large variation in daily temperature during winter. Additionally, in subtropical climate zones, the facilities for herds such as heating equipment and building insulation do not appear to be sufficient.

Weaning-to-mating interval (WMI)

The WMI is a reproductive performance measurement associated with PBA, FR and returns. Sows with a short WMI that are bred between 3 and 6 days after weaning have higher FR and PBA than those bred between 7 and 20 days post weaning (Hoshino and Koketsu, 2008; Tummaruk et al., 2010). The WMI tends to be increased by short lactation length and low feed intake during lactation (Koketsu et al., 1996b). In addition, prolonged WMI is related to a short duration of estrus and a shorter interval between onset of estrus and ovulation (Weitze et al., 1994; Kemp and Soede, 1996). A consequence of this is an increased risk of inseminating at a suboptimal period, which can be a major cause of low FR and low PBA. As previously mentioned, the use of a GnRH antagonist given to sows intravaginally facilitates a single dose fixed-time insemination in weaned sows. If this practice becomes common, WMI may become a less important factor for reproductive performance.

Number of pigs born alive (PBA)

The PBA in parity 1 is a factor that can help producers to identify high prolific sows at an early stage (Iida et al, 2015). A sow's PBA is determined by environmental or management factors and genetic potential (Hoving et al., 2011). Sows that have a high PBA in parity 1 typically produce high PBA throughout all the subsequent parities, and have high FR up to parity 3. These high prolific sows also have high lifetime reproductive performance.

Birth weight and preweaning growth rate

Litter-of-origin in sows includes their birth weights and preweaning growth rate. Gilts with lower age at puberty have heavier birth weights and higher preweaning growth (Vallet et al., 2016). These characteristics may affect subsequent reproductive performance of sows. Preweaning growth is affected by sow milk production, whereas heavier birthweights are associated with fewer pigs born in the litter.

Number of pigs weaned

An increased number of pigs weaned or heavier litter weight at weaning could impair a sow's post weaning reproductive performance due to increased loss of body reserves in the lactating sow. Therefore, the use of fostering and nurse-sow techniques impairs the metabolic state of sows and decreases post weaning reproductive performance (Quesnel et al., 2007). Also, sows that fostered 3 or more piglets have been found to have prolonged WMI (Usui and Koketsu, 2013).

Age of gilts at first-mating (AFS)

Gilt development and management is a critical to optimize the reproductive performance of sows. It is useful to record age of gilts at first estrus and dates of heat-no-serve in gilt development and management. However, the age of gilts at first estrus and dates of heat-no-serve are hardly recorded in commercial swine herds in North America, whereas AFS is commonly recorded (Patterson et al., 2010). Therefore, AFS in herd data analysis is still a factor for PBA and lifetime performance in commercial herds.

Increased AFS is associated with increased PBA in parity 1 (Iida et al., 2015). In the U.S.A., southern E.U. and Japan a typical AFS of approximately 240 days has been practiced to increase body weights and more body reserves of replacement gilts to be first-mated.

Number of stillborn piglets

By definition, stillborn piglets are those piglets that are alive at the initiation of farrowing but die intrapartum (Dial et al., 1992). In practice, the stillborn piglets in commercial herds are categorized as piglets found dead behind the sow at the first check up after parturition, with no sign of decomposition (Vanderhaeghe et al., 2013). Like AFS or WMI, the number of stillborn piglets is a factor in sow performance and is related to other aspects of reproductive performance. For example, abortion risk for sows has been found to be associated with sows having stillborn piglets (Iida et al., 2016).

Keynote Speakers

Such an association between an increase in abortions and the number of stillborn piglets could be explained by having infectious agents, such as porcine parvovirus, and also porcine reproductive and respiratory syndrome virus (Almond et al., 2006).

D. Herd-level information

Herd-level information includes various useful factors that can be used to characterize a production system. Herd characteristics, management practices, production systems and facility types can all be analyzed as herd level information.

Herd size

Herd size is an indicator of how advanced a production system is, including the amount of investment and the quality of the facilities and human resources and the level of genetic improvement. Larger herds were associated with high PWSY (King et al., 1998). The herd size itself does not appear to directly increase PWSY, but large herds tend to be able to hire more specialized workers and use better facilities than small herds. Also, there may be more rapid genetic improvement and a better production system in larger herds.

High-performing herds

The concept of high-performing herds is related to best-practice benchmarking, which has been used to provide values for target performance and efficiency (Koketsu, 2007). Herds can be categorized into two herd categories based on PWSY: high-performing herds and ordinary herds. In southern European countries, high-performing herds have 4-7% higher FR and 4-6% lower return risks across parity than ordinary herds. Consequently, these high-performing herds have fewer non-productive days, such as reservice interval and culling interval. Also, the high-performing herds have 0.6-0.9 pigs more PBA and 0.8-0.9 more pigs weaned across parities than ordinary herds. With regards to culling management, the high-performing herds have lower culling rates from parities 0 to 5 but higher culling rates in parity 6 or higher than ordinary herds.

Herd management factors

Information relating to herd management factors can be collected by the means of a questionnaire survey. The survey can collect information about gilt development programs, insemination timings, farrowing and lactation management, farrowing spaces and culling guidelines and so on. Analysis of such herd management information shows that herds performing first insemination immediately after first detection for gilts, or within 6-12 hours for sows, had higher FR than those with later times for insemination (Kaneko et al., 2013). Furthermore, the analysis showed that AFM of gilts in the herds using direct boar contact was 14 days less than that in the herds using indirect contact (Kaneko and Koketsu, 2012). Another finding from herd management analysis is that actual culling intervals for mated gilts and sows were at least 30 days longer than the guideline culling interval (Sasaki and Koketsu, 2012).

Within-herd variability for number of mated females or age structure

A consistent flow of pigs through a production facility becomes more important as production systems become more standardized. Using a group measurement of females in breeding herds, within-herd variability in the flow of pigs in a breeding operation can be measured as the number of females mated per week, over a 52-week period. Large within-herd variability in the number of females mated 16-19 weeks previously is associated with lower annual FR, increased non-productive days and decreased herd reproductive productivity (Koketsu et al., 1999) and farrowing space utilization (Koketsu et al., 2015).

Herds that have a stable age structure over 2 years have higher FR than those in herds having an unstable age structure (Koketsu, 2005a). This is because the herds with a stable age structure had higher proportions of parities 3-5 sows and a lower proportion of gilts than the herds with an unstable age structure. Therefore, within-herd variability affects efficiency in breeding herds.

Number of farrowing spaces

A limited number of farrowing spaces is a bottleneck for pig production in most breeding herds. A recent study showed that sows in high-performing herds, based on the number of pigs weaned per farrowing space per year, produced 130 (+ 3.5) pigs and 836 (+ 2.3) kg (Koketsu et al., 2015). A higher farrowing space utilization efficiency is associated with lower within-herd variability measured as the coefficient of variation (%) for the number of females mated 16 weeks previously.

Boar and semen factors

Semen characteristics including motility parameters may affect reproductive performance of sows. However, data about semen characteristics in boar studs have not been combined well with sow performance data in commercial herds (personal communication from Dr. Neil DeBuse, U.S.A.). There needs to be more research integrating field data on boar semen quality with reproductive performance of sows in order to identify the causes of poor performance at boar, sow and herd levels, and also to determine the motility parameters and the optimum number of motile cells in a dose (Broekhuijse et al., 2011).

E. Limitations of data analysis using commercial herd data

There are certain production research areas which can be investigated using epidemiological studies or which are suitable for situations that would require excessive funding or time to conduct in controlled experiments. As information technology advances, production research can evolve by using commercial herd data to disseminate useful information for producers and veterinarians.

However, there are several limitations with non-controlled observational studies that would not occur in controlled experiments led by university researchers. For example, some commercial herd data that are recorded incorrectly, and this means that some exclusion criteria are essential. Also, herd health, nutrition, management practices and genotype may not be well controlled in observation studies. Additionally, sows are not randomly selected and multiple observations per sow are not independent units of observation. One other limitation is that herds' data are also in a two-level structure because management practices, production systems, facilities and herd health programs vary between herds, i.e., sows are not independent of the herd. Even with such limitations, herd data analysis using appropriate exclusion criteria and multi-level statistical models can disseminate practical and readily applicable information to swine veterinarians and producers about production issues that are difficult to be investigated by controlled experiments.

Keynote Speakers

Conclusions

It is critical for veterinarians to know about the factors affecting reproductive performance in order to optimize their clients' breeding herd productivity. Improving the herd management that controls these factors, together with genetic improvement, will enable us to reach 40 PWMSY. Finally, in order to empower data analysis it is necessary to ensure correct data recording, data collection and data integrity checks.

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Keynote Speakers

How to handle Swine Colibacillosis in the field? What kind of resistance do we have to expect?

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Introduction

Colibacillosis is generally defined the infection with Escherichia coli characterized by many clinical forms. E. coli is a gram negative peritrichously flagellated bacteria belonging to the family Enterobatteriaceae and is the causative agent of a wide range of diseases in pigs, including neonatal diarrhoea, post-weaning diarrhoea (PWD), oedema disease (ED), septicaemia, polyserositis, coliform mastitis (CM) and urinary tract infection (UTI) (Fairbrother and Gyles, 2012).

Enteric colibacillosis may result in significant economic losses due to mortality, decreased weight gain, cost for treatments, vaccinations and feed supplements. In severe outbreaks of PWD a mortality rate up to 25% was reported (Fairbrother and Gyles, 2012). Depending on the severity of the disease, the cost of PWD was estimated to range from 40 to 314 per sow (Sjölund et al., 2014). Two main pathotypes are involved in enteric colibacillosis: enterotoxigenic E.coli (ETEC) and enteropathogenic E.coli (EPEC). ETEC is the most important pathotype in pig and include different virotypes (this term is used to describe strains characterized by different combinations of toxins and fimbriae). ETEC elaborate one or several enterotoxins that induce secretory diarrhoea, causing some of the most significant diseases in the pig industry worldwide, such as neonatal colibacillosis and PWD, the main subjects of this paper.

ETEC responsible of neonatal diarrhoea posses adhesins, surface proteins called fimbriae, identified as F4 (k88), F5 (k99), F6 (987P) and F41. The fimbriae allow the microorganism to adhere to specific receptors on the brush borders of the enterocytes of the small intestine. Growing up, piglets show a modification of their intestinal receptor for ETEC fimbriae and, as a consequence, their susceptibility to ETEC virotypes. For this reason PWD is most commonly caused by ETEC that usually have either F4 or F18 fimbriae (even though some exceptions are observed). A non-fimbrial adhesin identified as AIDA (adhesin involved in diffuse adherence) has been associated with ETEC strains recovered from weaned pigs with PWD and there is evidence that it plays a role in colonization of the intestine of pigs.

In general, ETEC adhere to the intestinal mucosa and produce enterotoxins causing changes of the flux of water and electrolytes in the enterocytes of the small intestine leading to diarrhoea when the excess of fluid in the lumen of the small intestine is not adsorbed in the large intestine. Excessive secretion and diarrhoea leads to dehydration, metabolic acidosis and death. Most ETEC of neonatal enteric colibacillosis produce heat-stable enterotoxin called STa. Based on the concentrations of the STa receptors, the posterior jejunum is the major site of hypersecretion in response to STa. ETEC strains responsible of PWD produce one or more of the enterotoxins STa, STb, LT for which the role in the development of diarrhoea has been defined. Enteroaggregative E.coli heat-stable enterotoxin (EAST1) was reported in ETEC isolated from pigs with diarrhoea, however its role in the development of diarrhoea has not been elucidated (Fairbrother and Gyles, 2012).

Managing enteric colibacillosis in pigs requires an understanding of the pathotypes and the virotypes of E.coli involved, as briefly reported above, and the conditions under which they are capable of causing disease, in order to implement appropriate diagnostics and strategies for prevention and control.

This paper is aimed at addressing some of the major questions that are frequently asked when facing with colibacillosis in the field, namely the diagnostic approach and the interpretation of the specific investigations, the epidemiology of the infection, the predisposing environmental conditions and host factors and the measures of prevention and control.
An accurate diagnosis is the key element for approaching a clinical problem. Treatment and control programs for enteric diseases are pathogen specific and only if the "enemy" is known, appropriate measures of control can be implemented. Control measures are traditionally based on the use of antimicrobials for prophylactic, metaphylactic and therapeutic purposes. In particular, preventive feed medication is still applied in many countries despite others have significantly reduced or eliminated these wide range treatments, as consumer resistance and the pivotal issues associated with the selection of resistant bacteria. Antimicrobial resistance is a major problem in veterinary medicine and represents a treat to public health. For these reasons strategies for control of enterotoxigenic E.coli will be considered in the paper, focusing mainly on antibiotic therapy and antimicrobial resistance.

The diagnosis

1. The diagnostic approach to neonatal Colibacillosis and post-weaning diarrhoea in pig

When an outbreak of diarrhoea occurs in a pig herd, the starting point for a rational implementation of the control measures is the achievement of a diagnosis. The presumptive diagnosis is based on clinical presentation and the gross lesions.

Neonatal diarrhoea due to E.coli is observed most commonly in piglets aged from 0 to 4 days of life, and in general, in an endemic condition, litters from first-parity sows could be more involved, due to a lack of protection by colostral immunity.

PWD due to E.coli is commonly observed 2-3 weeks after weaning, even if not exceptionally it can be recorded at 6-8 weeks after weaning.

When E.coli sustains the enteritis, diarrhoea is characterized by yellowish, gray or slightly pink fluid with a characteristic smell, lasting in general one week.

Necropsy can provide useful information to orient the diagnosis, although it is not reliable based solely on the correlated findings. The most effective approach is to select a number of untreated pigs (3-5) suffering from diarrhoea since less than 12-24 hours (acute phase), to humanely euthanize them and perform an accurate necropsy in order to evaluate gross lesions (evaluating small intestine, colon, ileo-caecal valve, mesenteric lymph-nodes). Autolysis of the gut after death occurs promptly so also recently spontaneously death animals cannot be informative.

The small intestine is usually dilated, slightly oedematous and hyperaemic and the dilated stomach shows hyperaemia in the fundus. These lesions, even not pathognomonic are suggestive of enteric colibacillosis.

Samples of small (in particular ileum and jejunum) and large intestine should be taken for bacteriological and virological investigations and fixed in 10% buffered formalin. In fact, the definitive diagnosis requires the combination of several investigations. The identification of E.coli infections is based on bacteriological examination of samples of luminal content (first choice) or rectal swabs. The samples should be inoculated onto blood agar and McConckey agar or other media which are selective for Enterobatteriaceae such as Hektoen agar. These selective media allow differentiation of lactose fermenting (such as E.coli) from lactose non-fermenting Gram negative enteric bacilli. Colonies on solid media reach their full size within 1 day of incubation and vary from smooth to rough or mucoid. The characteristics of the colonies grown on blood agar and lactose fermentation on selective media gives a first diagnostic indication. In particular the presence of haemolytic colonies, both in neonatal diarrhoea and PWD, is often used as a rapid tool for the diagnosis of ETEC diarrhoea. This finding allows also a preliminary assessment on the fimbrial type involved, since colonies of F4 and F18 positive ETEC strains are almost always haemolytic on blood agar, while F5, F6, F41 and EPEC are not haemolytic (Fairbrother and Gyles, 2012). The interpretation of the results obtained with the bacteriological examination in treated animals is in many cases unreliable.

Keynote Speakers

2. What are the next steps to reach a definitive diagnosis?

The E.coli strain, after the isolation on culture, should be identified as pathogenic, commonly by serotyping or genotyping. Serotyping is performed by tests of agglutination using polyvalent or monovalent sera to determine O (cell wall LPS) or F (fimbrial) antigens. In particular the determination of O antigens can be useful for diagnostic purposes since a small number of specific O groups have been associated with disease (table 1) (Fairbrother and Gyles, 2012).

Table 1: Important adhesins and serogroups of ETEC (modified from Fairbrother and Gyles, 2012).

ETEC Adhesins	O serogroups	Disease
F5, F6, F41	O8, O9, O20, O64, O101	Neonatal diarrhoea
F4	O8, O138, O141, O145, O147, O149, O157	
F4, AIDA	O8, O138, O139, O141, O147, O149, O157	PWD
F18, AIDA	O8, O138, O139, O141, O147, O149, O157	

Slide agglutination, for example, is a quick and cheap method commonly used in the past for the identification of F4 positive ETEC. This serotyping approach can lead to false classifications, mainly because of cross-reactions or lack of expression of fimbriae in vitro. For these reasons a complete serotyping of H (flagellar protein antigen) and O antigens, with the additional identification of K antigens (capsular polysaccharide), is the standard method for the definition of all serotypes, but in general is carried out in few reference laboratories (Fairbrother and Gyles, 2012).

Currently, genotypic analysis such as the polymerase chain reaction (PCR), for the detection of genes encoding for virulence factors is performed in many laboratories to characterize the strains isolated. Primers recognising different genes encoding for toxins (STa, STb, LT and EAST1) and fimbriae (F4, F5, F6, F18, F41) of ETEC, for the outer membrane protein Eae or intimin in enteropathogenic E.coli (EPEC) and for Stx2e toxin in STEC (E.coli strains involved in oedema diseases) strains, are available and can be used to perform PCR assays for daily routine diagnostic. Interestingly, certain F18 strains produce both enterotoxins and Stx2e. These strains are classified ETEC rather than STEC, since they produce clinical PWD more than oedema disease.

Histopathology in formalin-fixed, paraffin embedded tissue (ileum, jejunum and large intestine should be included) can be used as additional investigation for a definitive identification of E.coli that are observed adhering to the enterocyte brush border membrane of intestinal mucosa. E.coli F4 positive usually adhere to the cells of most of the jejunum and ileum, while other ETEC mainly colonize the distal jejunum or the ileum. Other changes include vascular congestion, haemorrhages and increased number of inflammatory cells (neutrophils and macrophages in the lamina propria and mild villous atrophy) (Fairbrother and Gyles, 2012). Interestingly, in EPEC infection the regions involved are usually the sides of the villi of the small intestine. The lesions are usually sparse and up to seven sections of intestine must be screened to observe the characteristic attaching and effacing (A/E) lesions.

3. Which differentials diagnosis should be considered in case of neonatal colibacillosis and PWD?

Disease outbreaks in large populations are multifactorial and focusing the diagnosis, as well as the following control strategies on single causes can misguide practitioners. The approach to enteric colibacillosis must consider the differential diagnosis and the potential different causes that can be involved at the same time in an outbreak. The predominant type of enteritis and the localisation of the lesions (small or large intestine, disseminated) are usually enough to reduce the potential causes to be listed in the diagnostic pathway. Diarrhoea in the pre-weaned piglet is probably straightforward to identify, treat, and prevent than post-weaning diarrhoea. In particular ETEC neonatal diarrhoea must be differentiated from other causes of diarrhoea, including EPEC, Clostridium difficile, Clostridium perfrigens type A and C, enteric coronavirus (TGEV, PEDV) and rotavirus groups A, B and C. In piglets older than 7 days coccidiosis due to Isospora suis should be also considered (Table 2).
ETEC PWD should be differentiated from other causes of diarrhoea already described in piglets such as EPEC, enteric coronavirus (TGEV, PEDV), rotavirus groups A, B and C, salmonellosis, proliferative enteropathy due to Lawsonia intracellularis and Brachyspira spp. (Table 3).

Table 2: Differential diagnosis of the main agents of neonatal diarrhoea (modified from Martelli et al., 2013).

Cause	Age	Diarrhoea	Gross Lesions	Mortality	Diagnosis
E.coli (ETEC, EPEC)	Most commonly from 0 to 4 days	Yellowish, gray or slightly pink alkaline pH	Distension, congestion of small intestine Stomach full of curdled milk	Can reach 70%	Culture/isolation Typing of isolates usually by PCR Histopathology
C.perfrigens type C	PA: 1 days A: 3 days SA: 7 days C: 10-14 days	PA: watery yellowish bloody A: brown bloody SA: watery grey/yellow C: yellow/grey	Jejunum and ileum mostly involved Haemorrhagic enteritis Bloody ascitis	100% in PA and A forms	Culture/isolation Typing/toxin identification Histopathology
C.perfrigens type A	Generally diarrhoea is observed within 48 hours of birth	Mucoid, pink without blood	Jejunum and ileum mostly involved Pasty content Presence of necrotic membrane	Generally low if not complicated	Culture/isolation Typing/toxin identification Histopathology
Clostridium difficile	In the first week of life	Pasty and yellow	Mesocolon oedema Typhlocolitis with focal erosions	Variable. Up to 50%	Culture/isolation Toxin identification
Coronavirus PEDV TGEV	All	Watery yellow/white/grey Watery yellow, white, grey, greenish; acid pH	Empty stomach Small intestine was thinned and congested	80-100% (1 week of age). Increasing the age decrease mortality	PCR Histopathology Viral isolation
Rotavirus	From 1 to 5 weeks	Watery, sometime pasty. Acid pH	Small intestine was thinned. Milk in the stomach	Low, < 20%	PCR Histopathology Viral isolation
Isospora suis	Not before 5 days More frequent around 14 days	Yellow and pasty. Alkaline pH	Small intestine. Enteritis with fibrino-necrotic membrane	Very low or not observed	Microscopic evaluation after flotation

PA: per-acute; A: acute; SA: sub-acute; C: chronic

Keynote Speakers

Table 3: Differential diagnosis of the main agents of post-weaning diarrhoea (modified from Martelli et al., 2013).

Cause	Age	Diarrhoea	Gross Lesions	Mortality	Diagnosis
E.coli (ETEC, EPEC)	Most commonly from 0 to 4 days	Yellowish, gray or slightly pink alkaline pH	Distension, congestion of small intestine Stomach full of curdled milk	Can reach 70%	Culture/isolation Typing of isolates usually by PCR Histopathology
C.perfrigens type C	PA: 1 days A: 3 days SA: 7 days C: 10-14 days	PA: watery yellowish bloody A: brown bloody SA: watery grey/yellow C: yellow/grey	Jejunum and ileum mostly involved Haemorrhagic enteritis Bloody ascitis	100% in PA and A forms	Culture/isolation Typing/toxin identification Histopathology
C.perfrigens type A	Generally diarrhoea is observed within 48 hours of birth	Mucoid, pink without blood	Jejunum and ileum mostly involved Pasty content Presence of necrotic membrane	Generally low if not complicated	Culture/isolation Typing/toxin identification Histopathology
Clostridium difficile	In the first week of life	Pasty and yellow	Mesocolon oedema Typhlocolitis with focal erosions	Variable. Up to 50%	Culture/isolation Toxin identification
Coronavirus PEDV TGEV	All	Watery yellow/white/grey Watery yellow, white, grey, greenish; acid pH	Empty stomach Small intestine was thinned and congested	80-100% (1 week of age). Increasing the age decrease mortality	PCR Histopathology Viral isolation
Rotavirus	From 1 to 5 weeks	Watery, sometime pasty. Acid pH	Small intestine was thinned. Milk in the stomach	Low, < 20%	PCR Histopathology Viral isolation
Isospora suis	Not before 5 days More frequent around 14 days	Yellow and pasty. Alkaline pH	Small intestine. Enteritis with fibrino-necrotic membrane	Very low or not observed	Microscopic evaluation after flotation

A: acute; C: chronic

4. Is the presence of intestinal ETEC sufficient to cause enteric colibacillosis?

Clinical manifestations of enteric colibacillosis obviously require the presence of enterotoxigenic E.coli but also environmental changes and recognized risk factors (Laine et al., 2008). Moredo et al. (2015) demonstrated that the percentage of ETEC positive non-diarrhoeic pigs was 16.6% during the lactation period, 66% in the nursery phase and 17.3% in the finisher population. These data demonstrates that this pathogen can also be shed in faeces from healthy animals as already reported by Osek, in 1999. This information must be considered for correct interpretation of diagnostic results. In particular the evaluation of diagnostic findings should be made only in consideration of both clinical signs and pathological lesions, as well as taking in account the concentration of the E.coli strain isolated, belonging to the identified pathotype and virotype.

Studies about phenotypic characterization of intestinal bacteria of pigs during suckling and postweaning period demonstrated that E.coli, streptococci of Lancefiled group D and K and Clostridium perfrigens are among the earliest bacteria to colonize the gut in piglets. These bacteria are considered normal inhabitants of the intestinal tract, with specific strains being an important cause of diarrhoea. The mean number of E.coli biochemical phenotypes in piglets increased as animals aged. After weaning the change of intestinal environment of piglets, mainly due to dietary changes, results in an alteration of the composition of the indigenous flora. The diversity of E.coli strains of intestinal flora is usually high in healthy pigs, while in enteric colibacillosis we observe an alteration of the balance between the bacteria present in the normal intestinal flora (Katouli et al., 1995). This condition lead to the proliferation of a dominating pathogenic strain, which colonize the small intestine (Hampson et al., 1988), rapidly reaching massive numbers to the order of 109/g of contents. This is the reason why frequently, if not always, samples collected in diarrhoeic pigs affected by colibacillosis, in the acute phase of the disease, allow the isolation of a pure culture of pathogenic E.coli.

5. Is the isolation of the pathogenic strain essential? What about the direct demonstration of virulence factors in the pathological samples (faeces, intestine, rectal swabs)?

Culture of the small intestine, faeces or rectal swabs of diarrhoeic pigs affected by enteric colibacillosis, as already mentioned, usually yields pure or nearly pure cultures of haemolytic or non haemolytic E.coli. ETEC isolated from cases of neonatal colibacillosis can appear as haemolytic (ETEC F4 positive) or non haemolytic (ETEC F4, F5, F6, F41) colonies on blood agar plates. ETEC isolated form cases of PWD are mostly haemolytic (ETEC F4 or F18) even if non haemolytic strains can be observed. For these reasons, although the presence of haemolytic colonies is frequently used as a rapid means for confirming a presumptive diagnosis of enteric colibacillosis, in particular in the post weaning period, the identification of virulence factors by PCR of E.coli isolates is of fundamental importance in order to correctly identify the pathogen. The use of PCR for the identification of virulence factors directly in samples from diseased pigs, without typing of individual isolates, can make the interpretation difficult and unreliable. This diagnostic approach, in fact, can give a mix of all the detectable virulence factors belonging to different E.coli strains present in the sample and, as a result, false combinations of these factors. In addition the presence of virulence factors of other intestinal Enterobacteriaceae, possessing similar genes, can be detected.

Usually outbreaks of F4 positive E.coli tend to involve only one strain at any one time, even if mixed infections with the isolation of different virotypes in the same outbreak were observed. In these cases probably one virotype predominates in any given outbreak (Fairbrother and Gyles, 2012). For these reasons in order to determine if more than one virotype is involved in an outbreak of enteric colibacillosis, it would be useful, compatibly with the costs for the examinations, to type more than one isolate (it might be advisable to test samples from 5 pigs with diarrhoea and typing 3 isolates previously chosen for their cultural and biochemical characteristics). Although this approach does not give absolute results, certainly it increases the reliability of the results obtained.
Back to the importance of the isolation of the strain responsible of the outbreak, this is a condition necessary to test in vitro the pathogen to different antimicrobial, in order to address veterinarians in the definition of an appropriate antimicrobial therapy.

6. Which methods are commonly used to evaluate the susceptibility to antibiotics? Do some molecules require specific methods for being tested? What about the interpretative criteria?

Two methods are mainly used to evaluate the susceptibility of bacteria to antibiotics: the disc diffusion method and the dilution susceptibility testing method.

The disc diffusion (Kirby-Bauer) method is widely used for antimicrobial susceptibility testing during the routinely diagnostic activity, it give a qualitative result and it is a quick and cheap method to evaluate the susceptibility of bacteria to antibiotics. Scientific organizations such as the Clinical Laboratory Standard Institute (CLSI) an international, interdisciplinary organization, promote accurate antimicrobial susceptibility testing (AST) and appropriate reporting by developing standard reference methods and interpretative criteria for the results of standard AST methods. Interpretative criteria of CLSI are developed based on international studies and are revised frequently. The disc diffusion

Keynote Speakers

test is not always a reliable method for detection of antimicrobial resistance. This is the case of colistin for which other methods, such as dilution-based methods (agar dilution test, broth dilution test, etc.), should be used (CLSI, 2008).

Dilution susceptibility testing methods are used to determine the minimal concentration of antimicrobial to inhibit (MIC) the microorganism, giving a quantitative result. This can be achieved by dilution of antimicrobial in either agar or broth media. The interpretation of the results is performed using clinical breakpoints (a discriminating concentration used in the interpretation of results of susceptibility testing to define isolates as susceptible, intermediate or resistant) developed again by Scientific Organization such as CLSI.

Interpretative breakpoints for susceptibility are described by clinical breakpoints that guide to the therapy, and epidemiological cut off. The epidemiological cut-off value (ECOFF) is the minimal inhibitory concentration/minimal effective concentration value that separates the bacteria population considered into those with and without acquired and/or mutational resistance based on their phenotypes (minimal inhibitory concentration). Any isolate presenting a MIC above this value is considered as resistant (non wild-type strain) irrespective of whether or not the achieved level of resistance compromises therapy. Isolates of a given bacterial species differing from the wild-type population might be considered resistant to the drug even if their MICs do not reach the clinical breakpoint, which predicts clinical success.

Back to colistin, the ECOFF calculated for E.coli is > 2 µg/ml (EUCAST 2009). The clinical breakpoints of colistin for Enterobacteriaceae have been reported by EUCAST (2013) and by the Comité de l'antibiogramme de la Société Française de Microbiology (CASFM 2014): susceptible ≤ 2 µg/ml; resistant > 2 µg/ml. These breakpoints are under continuous evaluation. Interestingly, Burch (2007) calculated that with feed concentration of 66 ppm, colistin can reach bactericidal concentrations, in the jejunum of pigs, for strains with MIC of 8 µg/ml, but not for strains of 16 µg/ml (Boyen et al., 2010).

The management of colibacillosis

1. What are the main preventive measures for neonatal colibacillosis?

Environmental temperature

One of the most important factors for preventing neonatal colibacillosis is the maintenance of piglets at an adequate environmental temperature. Ventilation should correctly create a dry and warm environment, reducing the moisture for bacterial growth. In particular sows need temperature not higher than 22°C, while a warmer creep area for piglets with constant temperature (30-34°C) must be considered (Fairbrother and Gyles, 2012).

Hygiene

Good hygiene in the farrowing crates reduces the environmental contamination by E.coli. This goal can be achieved through an all-in/all-out farrowing system and appropriate protocols of cleaning and disinfection of the farrowing room between batches.

Immunity

In general, most neonatal infections can be prevented by passive colostral and lactogenic immunity obtained by vaccination of the sow. However, as ETEC infections are non-invasive gastrointestinal infections, mucosal (i.e. lactogenic immunity) rather than systemic (i.e. colostral immunity) immunity will be important to fight the disease. Several killed whole cell bacterins or purified fimbrial vaccines are licensed to be administered parenterally in pregnant sow (Melkebeek al., 2013). Bacterins usually contain strains representing the most important serogroups and producing the fimbrial antigens F4, F5, F6 and F41, belonging to the virotypes most frequently responsible of neonatal diarrhoea. Vaccination is usually given parenterally at about 6 weeks and 2 weeks prior to parturition (Fairbrother and Gyles, 2012).

2. Which are the main preventive measures for ETEC PWD?

Good hygiene

Good hygiene in the farrowing area and nursery leads a reduction in the number of E.coli presented to the susceptible pigs. In particular, in PWD, environmental contamination with ETEC can be crucial for the infection of newly weaned pigs. Cleaning and sanitation are very important and in nursery with several weeks of production on the same site it is advisable additional measures other than the normal washing routine. In this case it can be recommended the use of NaOH soap, after the traditional cleaning (Rowles, 2014). This step provides additional impact on the removal of biofilm that can provide an excellent matrix for E.coli to reside and to resist to the disinfectants.

Sanitation of drinking water

Sanitation of water plays an important role too. Fresh, neutral or slightly acid drinking water, free of coliform and from high levels of iron, sulphates, magnesium, nitrates and manganese, contributes to prevent enteric diseases. Chlorination of drinking water is a cheap method to treat water lines and water systems even if it is not enough to remove the biofilm in water pipe. As a general rule, more chlorine will be needed to disinfect water with high levels of contamination (such as nitrite, iron, organic matter, etc.) or with a high pH, because the latter reduces the activity of chlorine.

Feeding regimen and dietary supplements

Restrictive feeding after weaning has been used as a preventive measure against PWD and overeating after weaning has been connected with the occurrence of PWD. In addition feed with decreased protein content was shown helpful against PWD outcomes. Reducing the nutritive value of the feed by increasing the fibre content and reducing crude protein and digestible energy is the first step for controlling PWD. Moreover, it is reported that the risk of PWD is higher in weaners fed only twice a day with restricted amount of feed than on the farms providing more than two meals per day with or without feed restriction or gave feed at libitum. So, low feed intake after weaning was considered a risk factor for diarrhoea in weaners (Laine et al., 2008).
Soybean-based feed was reported to favour PWD occurrence, but fermented soybeans or barley or rice diet can be of help in reducing PWD incidence.

Other supplements such as methionine, bovine lactoferrin peptides, glutamine, protease and lysozyme showed positive effect in PWD prevention. However, contradictory results are also reported in different studies, concluding that a reduced protein content level and diet supplementary intakes did not alter PWD outcomes.

Water acidification and organic acids

Normal water usually has a pH range of 7-8. To lower the pH of drinking water using for example organic acid, can reduce the faecal shedding of E.coli. Organic acids such as lactic, formic, propionic and acetic acid, contribute to the maintenance of the acid pH of the gastrointestinal tract, which may control potentially pathogenic bacteria. A study by Bosi et al. (1999) has shown that protected organic acid led to lower E. coli counts in the ileum and higher Lactobacillus counts in the colon indicating that protected organic acid is more effective in retarding absorption of dietary acids and allowing more effective delivery of acids to the distal ileum, caecum and colon of piglets. Medium chain fatty acids (MCFA) having 6 to 10 carbon atoms also have antimicrobial property. Dierick et al. (2002) reported that about 80% of the MCFA might exert bacteriostatic and bactericidal properties in the upper small intestine. Thus, it was assumed that including MCFA along with blends of organic acid would enhance its antimicrobial effects.

Keynote Speakers

Probiotics and prebiotics

Probiotic treatments with yeast or bacterial strains, for example belonging to the genus Lactobacillus, which competitively inhibit adherence of ETEC have been reported to have some beneficial effects against PWD. Also in this field contradictory results are shown by different studies. Saccharomyces has been reported to stimulate intestinal immunity and to inhibit binding of bacterial toxins to enterocyte receptors.
Prebiotics, such as selectively fermented ingredients, selectively stimulate the proliferation of potentially beneficial microorganism in the gastrointestinal tract.

Passive immunoprophylaxis

Feeding of spray-dried porcine blood plasma (SDPP) to pigs determine a reduction of the occurrence and frequency of PWD (Fairbrother and Gyles, 2012). This is probably due to the presence of specific anti-ETEC antibodies in the SDPP. It was demonstrated as these specific antibodies, which protect F4-receptor-positive pigs against ETEC infection, inhibit ETEC excretion and reduce the E. coli-induced inflammatory response of pigs (Bosi et al., 2004).
In the past it was reported that a certain degree of protection, against the F4 and F18 ETEC, was attained by the addition to the feed of eggs from hens immunized with specific antigens.

Active immunoprophylaxis

The passive protection decreases with aging and lactogenic immunity suddenly stops by weaning and as a consequence the newly weaned piglets become highly susceptible to enteric pathogens.
To prevent the colonization of PWD ETEC in newly weaned piglets, an F4 and/or F18 specific IgA response is needed in the small intestine at the moment of infection. The oral route is the most logical route to obtain this type of response. In order to have active acquired antibodies ready at weaning, piglets need to be immunized during suckling period, ideally 10 to 14 days before weaning (Melkeebek, 2013).

Several oral vaccines have successfully performed in weaned pigs using subunit vaccines as well as live oral vaccines. A live oral vaccine against F4 positive ETEC is currently commercialized in Canada, Brazil, Mexico and Europe. The vaccine can be delivered via drinking water or by individual drenching in piglets from 18 days of age. Seven days after vaccination, the onset of immunity is detectable and the duration of immunity is 21 days after the vaccine administration.

Unlike F4 fimbriae that induce protective anti-F4 immunity, oral administration of vaccines based on E. coli bacteria expressing F18 fimbriae or purified F18 fimbriae do not induce protective immunity against F18 ETEC. However, recent studies demonstrated that a minor subunit of F18 (FedF) induces protective anti-F18 antibodies and could represent a new approach for an anti-F18 vaccine (Zhang, 2014).

The vaccines currently available protect against F4 positive ETEC, but not against F18 ETEC, so the identification of E. coli responsible of colibacillosis outbreak is imperative for controlling the disease.
Although progress has been made in developing effective vaccines against PWD, the control of enteric colibacillosis is somewhat difficult due to the complexity of the disease and the immunological heterogeneity among ETEC strains. New approaches such as toxoid antigens, toxoid fusion antigens and multiepitope fusion antigen, should be considered in order to develop multivalent vaccines for effective protection against ETEC associated PWD (Zhang, 2014).

Zinc oxide

Zinc Oxide can be considered an alternative to antimicrobials or can be used in association. Feed containing between 2400 and 3000 ppm of zinc reduce diarrhoea, mortality and improve growth. For long, it has been thought that zinc oxide must have an antibacterial effect, especially against E. coli. Several antimicrobial mechanisms of zinc oxide were proposed: 1) hydrogen peroxide, which is generated from the surface of zinc oxide, can penetrate through the cell membrane, produce some type of injury, and inhibit the growth of the cells; 2) the affinity between zinc oxide and bacterial cells is an important factor for antibacterial activity. Other investigators showed that zinc oxide reduced bacterial adherence of ETEC F4 and blocked bacterial invasion by preventing increased tight junction permeability and modulating cytokine gene expression (Roselli et al., 2003).

Zinc is poorly absorbed, so it becomes highly concentrated in manure with implications in terms of environmental pollution. The therapeutic use of zinc is currently debated. In general terms bacteria in animals may develop resistance to Zn as well as to other heavy metals such as Cu. Resistance genes to Zn are often located on plasmids, which may be transferable to other bacteria, intra- and inter-species. Exposure to trace metals may also contribute to antibiotic resistance, even in the absence of antibiotics themselves. Zn supplementation to animal feed may increase the proportion of multi-resistant E. coli in gut microbiota (Yazdankhah et al., 2014). Several studies have focused the attention on heavy metals used in animal farming and possible mechanisms that could promote the spread of antibiotic resistance via co-selection. One report associated zinc with methicillin-resistant Staphylococcus aureus (MRSA) CC398 in Denmark (Aarestrup et al., 2010), concluding that zinc compounds may be partly implicated in the emergence of MRSA clones. The co-selection mechanisms include co-resistance and cross-resistance. Co-resistance is defined as the close proximity of two or more genetic elements encoding for resistances. Sulphonamide resistance, for example, would follow the co-resistance path. The cross-resistance evolves when an antibacterial agent attacks the same target, for instance efflux systems that simultaneously transport two or more types of antibacterial agents. An example of cross resistance could be done with tetracycline, as zinc resistant strains would also expel tetracycline using the same efflux system (Vahjen et al., 2015).

Antibiotic therapy and the prevention/treatment of enteric collibacillosis

1. What is the state of the art?

The use of antimicrobials is widely practiced for prophylaxis, metaphylaxis and therapeutic purposes in preventing and controlling pig enteric colibacillosis. Preventive feed medication is currently used in many Countries despite serious drawbacks and the oral administration of antibiotics is usually carried out in a large number of animals at the same time. Under-dosing is frequent with oral administration in pigs and this condition can favour the selection of resistant bacteria (Burrow et al., 2014). Antimicrobials commonly used to prevent or treat enteric colibacilosis must be chosen for their ability to achieve therapeutic concentrations at intestinal level. Among them, fluoroquinolones, cephalosporins, apramycin, ceftiofur, neomycin, amoxicillin/clavulanic acid, trimethoprim/sulphonamide and colistin are the most frequently used.

Antimicrobial resistance to several antibiotics such as apramycin, neomycin, trimethoprim-sulphametoxazole, and to colistin has been increasingly observed in ETEC strains causing PWD (Zhang, 2014). The development of resistance to a wide range of antimicrobial drugs, as well as the demonstrated trend of resistance in ETEC strains to the most of the antibiotics used for the treatment of colibacillosis in pig, is nowadays reason of concern (Luppi et al., 2013).

2. What about the resistance of E. coli to antibiotics reported in different countries?

It is difficult, if not impossible, to provide general data on resistance, because the situation is variable in different Countries and pig populations, and mainly depends on the antimicrobial preferentially used.

In a study performed in Italy (Luppi et al., 2013) on E.coli F4 positive, aiming to evaluate the trend of resistance of ETEC isolated in a period of 10 years (2002-2011), isolates obtained from cases of colibacillosis were tested using the disc diffusion method to several antibiotics. Isolates showed a statistically significant increasing trend of resistance over the whole period of study to: enrofloxacin (from 14.5% to 89.3%), flumequine (from 49.1% to 92.9%), florfenicol (from 9.8% to 64.3%), thiamphenicol (from 50% to 92%) and cefquinome (from 3.8% to 44%). An increasing resistance (not statistically significant) was also observed to gentamicin (from 63.6% to 85.7%), apramycin (from 61.8% to 82.1%), trimethoprim-sulphametoxazole (from 75% to 89.3%), tetracycline (from 97% to 100%) and erythromycin (from 92.4% to 100%).

Keynote Speakers

Resistance to enrofloxacin was described in E. coli strains isolated in Austria (Mayrhofer et al., 2004) and in Brazil, where nearly 30% of the isolates from cases of neonatal colibacillosis were resistant to this antibiotic (Costa et al., 2010). Fluorochinolones resistance has been strongly correlated with the quantity of the drug used to treat pigs and plasmid borne transfer of fluorochinolones resistance, in pig E. coli strains, has been demonstrated (Barton, 2014). Resistances to cefquinome and ceftiofur have been already described among E. coli isolates as demonstrated in a Swiss study (Stannarius et al. 2009). Relatively low levels of resistance to ceftiofur were reported in E. coli isolates from diseased pigs in Canada (11%) and Spain (4%) (Aarestrup et al., 2008). High levels of resistance to gentamicin were reported in pathogenic E. coli isolates in Belgium (46%), Poland (45%) and Spain (20%) (Aarestrup et al., 2008). Resistance to gentamicin and other aminoglycosides is usually transmissible, encoded on conjugative R-plasmids and often linked to resistance to other antimicrobials. The gene aac(3)-IV is the identified gene causing enzymatic cross-resistance between gentamicin and apramycin (Jensen et al., 2006).

Resistance to florfenicol is reported in pathogenic E.coli. The increased resistance to this relatively new molecule should be a reason of concern, even if differences are observed between countries and/or between pathogenic and commensal strains. As an example, in a study performed in Switzerland, low resistance prevalence was found for florfenicol as well as for amoxicillin, amoxicillin/clavulanic acid, ampicillin, cefquinome, ciprofloxacin, colistin and gentamicin in E. coli isolated from healthy weaner pigs. The most frequently found resistances were against streptomycin (60.6%), sulphonamide (51.5%), tetracycline (35.2%) and trimethoprim (27.5%). With exception of colistin, most resistances were found for those antibiotics commonly used on the farms (Stannarius et al., 2009). Resistance to trimethoprim-sulphamethoxazole and to tetracycline are frequently observed among E. coli isolates as reported in UK, Spain, Canada, Denmark, France and Japan (Burch, 2005; Stannarius et al., 2000; Kozak et al., 2009; Aarestrup et al., 2008). High levels of resistance to streptomycin (88.3%), trimethoprim/sulphonamide (78.8%), tetracycline (57.3%) were described in E. coli strains isolated from faecal samples derived from swine in Poland (Mazurek et al, 2013). The high levels of resistance to tetracycline is probably due to the wide use of this antibiotic in the past for treating pig respiratory and enteric bacterial diseases, as described by Burch in 2005 in the UK.

The majority of the E. coli isolates from cases of PWD in Australia resulted resistant to streptomycin and tetracycline and just less than an half were resistant to spectinomycin, ampicillin and trimethoprim-sulphametoxazole. In the same study, a smaller number of isolates were resistant to neomycin and apramycin, and a proportion of these showed resistance to gentamicin, while few were resistant to florfenicol. None of the isolates were resistant to enrofloxacin or ceftiofur (Smith et al., 2010). A study performed in Korea showed as E. coli strains isolated from diarrhoeic pigs were multi-resistant (resistant to more than 4 antibiotics) whit high levels of resistance to several antibiotics: streptomycin (100%), tetracycline (97.3%), gentamicin (77%), trimethoprim-sulphametoxazole (75.7%), amoxicillin (75.7%), ampicillin (73%), chloramphenicol (64.9%), enrofloxacin (64.9%) and ciprofloxacin (59.5%) (Lee et al., 2009).

Multidrug resistance among ETEC isolates has been described and recently there has been an increasing tendency for porcine ETEC to express a multidrug-resistant phenotype (Smith et al., 2010). Multidrug-resistant pathogenic E. coli strains are often isolated from diarrhoeic pigs and resistance genes are frequently on plasmids.

3. What are the prospects for the use of colistin in the treatment of colibacillosis?

Colistin is commonly used, mainly in oral presentation, due to its excellent activity against E. coli. Colistin is a bactericidal drug that binds to lipopolysaccharide (LPS) and phospholipids in the outer membrane of Gram negative bacteria. Studies on the use of antibiotics in France showed that one-third of the antimicrobials used in pigs was attributable to colistin. In Belgium colistin is the most commonly used antibiotic to treat PWD and oedema disease in pigs. High colistin use was reported in Spain, while in Denmark, its use increase from 2003 to 2011. The usually recommended dose for therapy is 100,000IU/kg BW, but in some non-European countries, it is authorised the use of colistin at lower dosage, as feed additive for growth promotion. Even if colistin was characterized by a general low degree of resistance, in the last few years E. coli strains resistant to colistin is becoming more common. Strains of E. coli with acquired resistance are encountered among pathogenic isolates commonly in pigs suffering of diarrhoea (Kempf et al., 2013).

Table 4: Percentage of colistin resistance in E.coli isolated from healthy and diseased pigs (modified from Kempf et al., 2013).

Country	Origin of the isolates	% of resistance/non-wild type strains	Reference
France	faeces, healthy pigs	0.5%	Belloc et al., 2008
Sweden	healthy pigs	0%	SVARM, 2011
Denmark	healthy pigs	0%	DANMAP, 2009
Belgium	pigs with diarrhoea	9.6%	Boyen et al., 2010
Croatia	pigs with diarrhoea	3%	Habrun B., 2011
Brazil	pigs with diarrhoea	6.3%	Morales et al., 2012
Brazil	pigs with diarrhoea	28.1%	Costa et al., 2010
UK	Slaughterhouse, healthy pigs	34,1%	Enne et al., 2008
China	pigs with diarrhoea	33.3%	Lu et al., 2010
Japan	pigs with diarrhoea	35.6%	Harada K., 2005
Italy	pigs with diarrhoea	42.2%	Data not published, 2015

Resistance to colistin is based on mutations responsible for modification of the LPS charge. Until now, polymyxin resistance has involved chromosomal mutations making the resistance mechanism unstable and incapable of spreading to other bacteria but has never been reported via horizontal gene transfer. A study performed in China (Liu et al., 2016) on antimicrobial resistance in commensal E. coli from food animals has shown an increase of colistin resistance and has described the emergence of the first transmissible, plasmid-mediated polymyxin resistance in the form of MCR-1. In terms of antibiotic resistance, plasmids play a central role as vehicles for resistance gene capture and subsequent dissemination.

Following a request from the European Commission (EC), in July 2013 the Committee for Medicinal Products for Veterinary Use (CVMP) and the Committee for Medicinal Products for Human use (CHMP) of EMA (the European Medicines Agency), adopted scientific advice and detailed considerations on colistin. This advice critically reviews recent information on the use of colistin in food-producing animals in the EU, its effect on the development of resistance to this category of antimicrobial agents in bacterial species that are of importance for human and animal health, and the possible impact on human and animal health.

The advice confirmed the importance of colistin in veterinary medicine for the treatment of enteric diseases in certain food-producing species for which there are few effective alternatives available. It was also highlighted that currently there is no evidence of spread of colistin resistance from food-producing animals to human patients, or vice versa. However, colistin is nowadays a last resort drug in human medicine in the context of treatment of infections caused by multi-drug resistant Pseudomonas aeruginosa, Acinetobacter baumannii and Enterobacteriaceae such as E. coli and Klebsiella pneumoniae, for which mortality can be extremely high. Based on current evidence, the advice concluded that it is considered appropriate to maintain the use of colistin in veterinary medicine but to restrict indications to therapy or metaphylaxis and to remove all indications for prophylactic use in order to minimise any potential risk associated with a broader use. The advice give indications also about combinations of colistin with other antimicrobials and unless valid justification, combination products should be withdrawn. The advice gives also other responsible use principles:

1. to avoid the use of colistin as a substitute for good management practices
2. colistin should be only used based on susceptibility testing
3. the duration of the treatment should be limited to the minimum time necessary for the treatment of the disease, and that treatment should not exceed 7 days (these indication should be added in the summary of product characteristics or SPC)
4. use of the product deviating from the instructions given in the SPC may lead to treatment failures and increase the prevalence of bacteria resistant to colistin.

Keynote Speakers

The final opinion was converted into a Decision by the European Commission on 16 March 2015. Recently the EMA has been asked by the European Commission to update its advice on the use of colistin in animals in the light of the recent discovery reported by Liu et al. (2016) described above.

Conclusion

Managing colibacillosis in pigs requires the understanding of the epidemiology of different pathotypes and virotypes and the conditions under which they are capable of causing disease. Before that, it is of fundamental importance to achieve a correct diagnosis and the laboratory is of great help to make a definitive diagnosis that allows the establishment of preventive and control measures. For these reasons routine diagnostics are needed to know what pathogens are involved in a hypothetic outbreak, in which the diarrhoea is the main symptom. A complete and accurate diagnosis considers an appropriate sampling for isolation, typing and testing the antimicrobial susceptibility of the pathogen. The use of antimicrobials is widely practiced for prophylaxis, metaphylaxis and therapeutic purposes. The growing concern about the increase of antimicrobial resistance among pathogenic E. coli strains is driving more attention to the alternatives to antibiotics such as vaccines, probiotics, prebiotics, additives, and good management practices. It is always more frequent to isolate multi-resistant E. coli strains from diarrhoeic pigs and in many cases there are very few alternatives, in terms of molecules that can be effective against these pathogens. These conditions force a more rational and judicious use of the antibiotics.

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Keynote Speakers

How to Deal with Success in Genetic Improvement

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There are several different genetic technologies that have been used to improve trait performance for pig farmers. Some examples would include:

a) Terminal Cross Mating System: this is simply using a crossbred female composed of breeds that are best for reproduction as the maternal line animals. The most common examples are the use of a Landrace/Large White F1 female as the parent female. This allows the best breeds for reproduction to exert their strengths, and takes advantage of the heterosis that is expressed by the F1 female for reproductive traits.

b) Optimal Genetic System Structure: this is where the production farm uses the proper number of GGP, GP and Parent Stock females in the genetic system. Usually at least 10% of the female herd should be in the GGP and GP roles. These maternal females then are mated to a breed or line of terminal boar that best fit the needs of the producer. The most common terminal breeds are the Duroc, Pietrain, or composite terminal lines. This allows for the producer to take advantage of both maternal heterosis in the females, and heterosis for terminal traits in the market hogs.

c) BLUP based selection programs: in order to maximize the genetic progress from selection, particularly in the maternal lines, we must use the most accurate method for estimating genetic merit in the animals eligible for selection. The BLUP system of breeding value estimation is the most accurate system available, and can be used by both the genetic supplier and the commercial producer to improve the traits of economic importance.

d) Marker Assisted Selection: we can now identify individual genes that have a major influence on certain traits of economic importance, such as litter size, feed conversion and meat quality. However, the genetic assay is somewhat expensive and is usually done by the genetic supplier, but the commercial producer is the one that enjoys the benefits.

e) Mate-Select Software: it is now possible for the genetic supplier to use software that can structure the matings made at the GGP level to produce the most extreme progeny from the pure line matings, while minimizing inbreeding. This is very important in the production of the most extreme boars for AI usage in both the maternal and terminal lines. This is usually employed by the genetic supplier and the results are then relayed to the commercial producer.

f) Genomic information: it is also now possible to genotype GGP animals for a large number of gene pairs, in excess of 60,000 genes for one animal. The cost of this is still quite high, but it can result in a significant increase in the accuracy of BV estimation, but due to cost, is employed at the genetic supplier only.

It is now possible for the commercial pork producer to reap the benefits from all the above genetic technologies, either directly using them, or having their genetic supplier use them.

In terms of improvement in performance of key swine traits, over the past 30 years we have seen:

1. Litter size has increased from 10 14 or more pigs/litter
2. Backfat has decreased from 25mm at P2 measurement 10mm or less
3. Lean meat percentage has increased from 45% 57% or more
4. Growth rate has increased from a 220 lb pig @ 7 months of age 285 lb pig @ 6 months of age
5. Feed conversion has improved from 3.50 lb feed/lb gain 2.50 lb feed/lb gain or less

While this is an overall positive change, in making genetic improvement we have to be aware of any possible 'correlated responses' to this genetic improvement in trait performance. Pearson (1903) and Robertson (1967) opined that "..individuals with intermediate trait values have higher fitness..", and, "..extreme phenotypes cause reduced fitness..".

For example, as litter size has increased, it has exceeded the uterine capacity of the sow, and as a result while more pigs are farrowed and weaned per litter, the number of piglets with low birth weights has increased. The potential impacts of this include an increased piglet mortality, and of those piglets that do survive to weaning have a higher percentage of pigs with sub-standard weaning weights.

Also, as backfat has decreased and lean meat percentage has increased and growth rate has increased, the percentage of animals that are more fragile and have more structural problems has increased. These negative correlated responses in fitness traits exert an economic impact with higher sow and pig mortality, higher sow culling rates, and an increased percent of sub-standard market hogs.

The overall goal of pork production is to maximize profit. So while there is a need to maximize the performance of key economically important traits in pork production, at the same time we must consider any potential correlated responses that might negatively impact profit.

Profit = Return > Cost of Production

Profit can be improved by either increasing the return from animals sold from the farm (by increasing reproduction, survival or growth), or by lowering the cost to produce the pigs (by improving feed conversion and reducing non-productive sow days). In order to maximize the profit the pig farmer needs to know their cost of production, including the specific components. Software can be utilized to assist the pig farmer in knowing their cost of production, and to examine the overall benefits from changing any of the components. The software demonstrated below is PigProfitTracker© developed by the Iowa Pork Industry Center at Iowa State University, Ames, IA.

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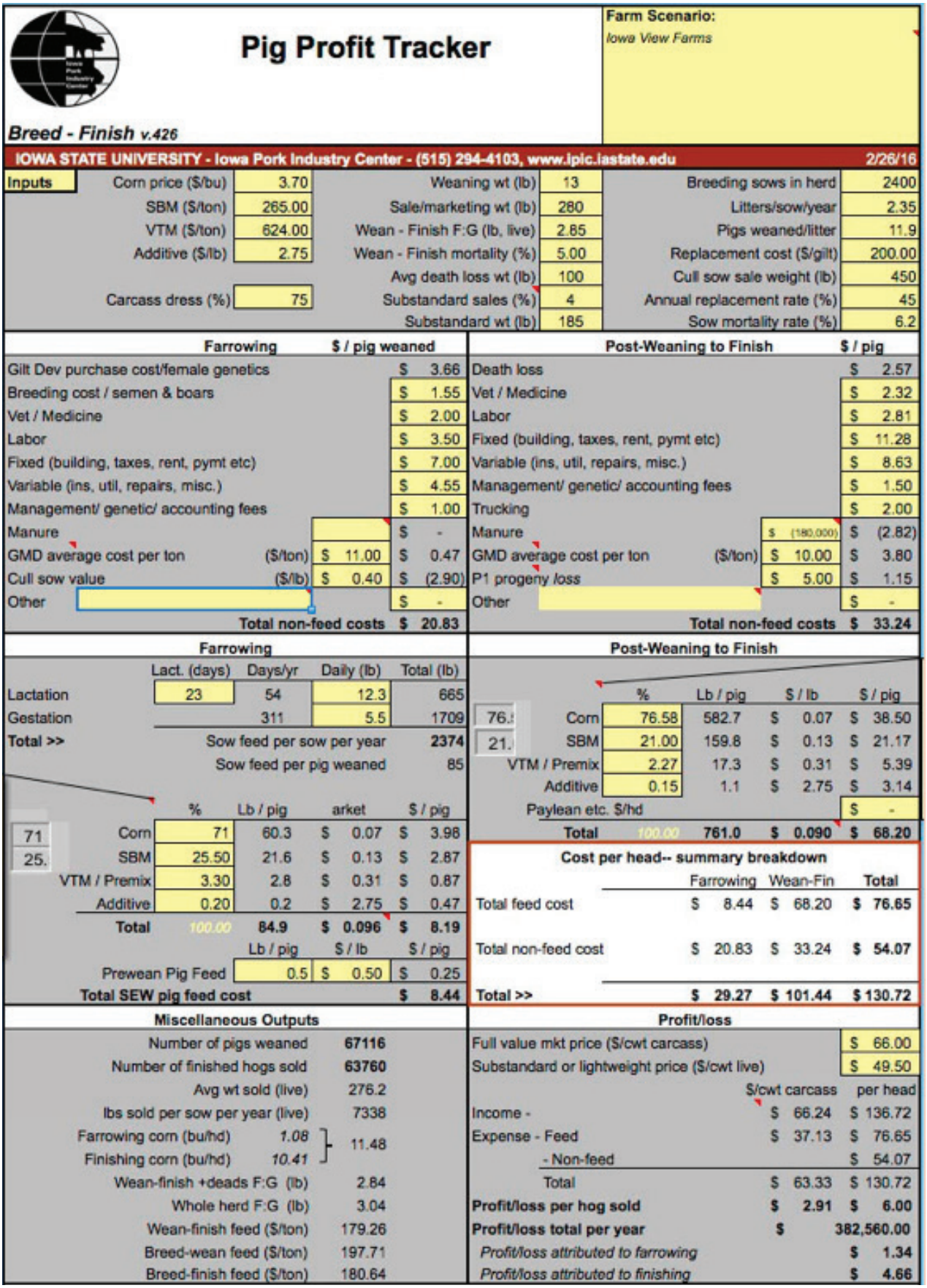
Figure 1: Example of cost of production/potential profit for use by swine producer.



Pig Profit Tracker

Farm Scenario: *Iowa View Farms*

Breed - Finish *v.426*

IOWA STATE UNIVERSITY - Iowa Pork Industry Center - (515) 294-4103, www.ipic.iastate.edu 2/26/16

Inputs							
	Corn price ($/bu)	3.70	Weaning wt (lb)	13	Breeding sows in herd	2400
	SBM ($/ton)	265.00	Sale/marketing wt (lb)	280	Litters/sow/year	2.35
	VTM ($/ton)	624.00	Wean - Finish F:G (lb, live)	2.85	Pigs weaned/litter	11.9
	Additive ($/lb)	2.75	Wean - Finish mortality (%)	5.00	Replacement cost ($/gilt)	200.00
			Avg death loss wt (lb)	100	Cull sow sale weight (lb)	450
	Carcass dress (%)	75	Substandard sales (%)	4	Annual replacement rate (%)	45
			Substandard wt (lb)	185	Sow mortality rate (%)	6.2

Farrowing			$ / pig weaned
Gilt Dev purchase cost/female genetics			$ 3.66
Breeding cost / semen & boars			$ 1.55
Vet / Medicine			$ 2.00
Labor			$ 3.50
Fixed (building, taxes, rent, pymt etc)			$ 7.00
Variable (ins, util, repairs, misc.)			$ 4.55
Management/ genetic/ accounting fees			$ 1.00
Manure			$ -
GMD average cost per ton	($/ton)	$ 11.00	$ 0.47
Cull sow value	($/lb)	$ 0.40	$ (2.90)
Other			$ -
		Total non-feed costs	**$ 20.83**

Post-Weaning to Finish			$ / pig
Death loss			$ 2.57
Vet / Medicine			$ 2.32
Labor			$ 2.81
Fixed (building, taxes, rent, pymt etc)			$ 11.28
Variable (ins, util, repairs, misc.)			$ 8.63
Management/ genetic/ accounting fees			$ 1.50
Trucking			$ 2.00
Manure		$ (180,000)	$ (2.82)
GMD average cost per ton	($/ton)	$ 10.00	$ 3.80
P1 progeny loss		$ 5.00	$ 1.15
Other			$ -
		Total non-feed costs	**$ 33.24**

Farrowing

	Lact. (days)	Days/yr	Daily (lb)	Total (lb)
Lactation	23	54	12.3	665
Gestation		311	5.5	1709
Total >>		Sow feed per sow per year		**2374**
		Sow feed per pig weaned		85

	%	Lb / pig	arket	$ / pig
71 Corn	71	60.3	$ 0.07	$ 3.98
25. SBM	25.50	21.6	$ 0.13	$ 2.87
VTM / Premix	3.30	2.8	$ 0.31	$ 0.87
Additive	0.20	0.2	$ 2.75	$ 0.47
Total	100.00	**84.9**	**$ 0.096**	**$ 8.19**

	Lb / pig	$ / lb	$ / pig
Prewean Pig Feed	0.5	$ 0.50	$ 0.25
Total SEW pig feed cost			**$ 8.44**

Post-Weaning to Finish

	%	Lb / pig	$ / lb	$ / pig
76. Corn	76.58	582.7	$ 0.07	$ 38.50
21. SBM	21.00	159.8	$ 0.13	$ 21.17
VTM / Premix	2.27	17.3	$ 0.31	$ 5.39
Additive	0.15	1.1	$ 2.75	$ 3.14
Paylean etc. $/hd				$ -
Total	100.00	**761.0**	**$ 0.090**	**$ 68.20**

Cost per head-- summary breakdown

	Farrowing	Wean-Fin	Total
Total feed cost	$ 8.44	$ 68.20	**$ 76.65**
Total non-feed cost	$ 20.83	$ 33.24	**$ 54.07**
Total >>	**$ 29.27**	**$ 101.44**	**$ 130.72**

Miscellaneous Outputs

Number of pigs weaned		**67116**
Number of finished hogs sold		**63760**
Avg wt sold (live)		276.2
lbs sold per sow per year (live)		7338
Farrowing corn (bu/hd)	*1.08*	11.48
Finishing corn (bu/hd)	*10.41*	
Wean-finish +deads F:G (lb)		2.84
Whole herd F:G (lb)		3.04
Wean-finish feed ($/ton)		179.26
Breed-wean feed ($/ton)		197.71
Breed-finish feed ($/ton)		180.64

Profit/loss

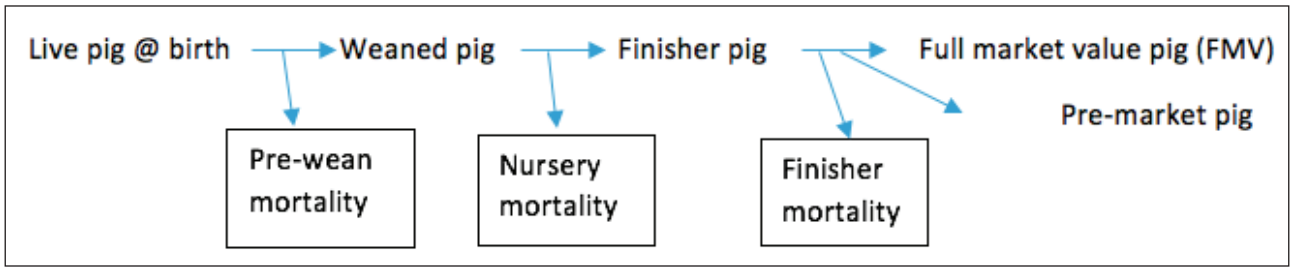
Full value mkt price ($/cwt carcass)		$ 66.00
Substandard or lightweight price ($/cwt live)		$ 49.50
	$/cwt carcass	per head
Income -	$ 66.24	$ 136.72
Expense - Feed	$ 37.13	$ 76.65
- Non-feed		$ 54.07
Total	$ 63.33	$ 130.72
Profit/loss per hog sold	**$ 2.91**	**$ 6.00**
Profit/loss total per year	**$**	**382,560.00**
Profit/loss attributed to farrowing		**$ 1.34**
Profit/loss attributed to finishing		**$ 4.66**

At the sow farm, the capacity is usually determined by the desired output of market hogs and level of investment of the farm owners. The correct number of gestation spaces, farrowing spaces and gilt development spaces is based on the overall desired production capacity. The primary duties are to breed the sows, farrow the sows, wean the litters, then repeat the process. Consequently then the sow farm costs accumulate on a sow/day basis. The owner must feed the sows, pay for the facilities, labor, vet-med costs, gilt development costs, genetic costs and other variable costs. The returns from the sow farm then come from full value weaned pigs, marginal value weaned pigs, and cull sows that are sold.

There then are a number of variables or 'traits' that can influence either the cost or return at the sow farm. Returns can be increased by increasing farrowing rate, increasing the number of pigs born alive (of adequate size), decreasing piglet mortality, increasing piglet weaning weight or decreasing sow mortality and cull rate. Costs can be reduced by reducing non-productive sow days and by decreasing piglet mortality. Non-productive sow days are decreased when farrowing rate is increased and wean to service interval is decreased. In order to make genetic improvement in these traits, they must have adequate genetic control to respond to selection, and the pig farmer must use the correct genetic system to get heterosis for these traits. The traits that have been shown to respond to BLUP based selection include litter size, pre-weaning mortality, litter weaning weight, and litters/sow/year (as a way to indirectly improve farrowing rate). Sow mortality can be improved by phenotypic selection in structure and soundness.

Economic Impact of Piglet Birth Weight:

I have used the term 'number born alive of adequate size' as a trait to be selected for. We have seen tremendous genetic progress in litter size since the implementation of BLUP based selection. However, as we have increased litter size above 12 NBA, the number of pigs with birth weights less than 1 kg has increased, along with piglet mortality. This is due to the litter size exceeding the uterine capacity of the sow, with pigs in large litters getting less pre-natal nutrients from the sow's uterus, resulting in more pigs that are smaller at birth than desired.
People who have raised pigs have long realized that the birth weight of a piglet is associated with it potential to make a profit for the pig farmer. The lifelong pathway of commercial pigs is shown in the diagram below:



Research has shown a strong relationship between the birth weight of a piglet and its survival rate, weaning weight, market weight, and potential profitability.

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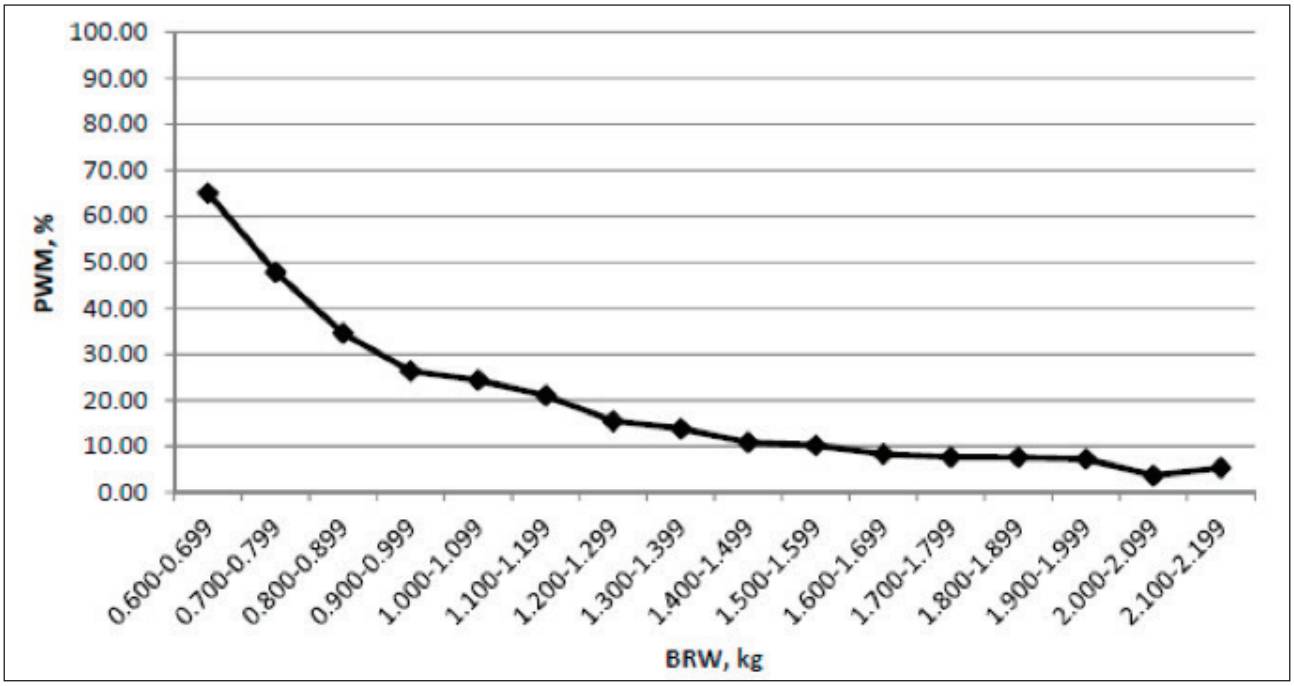
The tabe below shows the relationship between piglet birth weight and survival rate (Dufrasne et al, JAS 2013).



If the piglet has a birth weight in excess of 1.4 kg then the pre-weaning mortality is less than 10%. If the piglet has a birth weight of less than 1 kg, then the pre-weaning mortality is very high, in excess of 25%. Based on these results, it appears that a reasonable target for piglet birth weight of 1 kg and higher will keep pre-weaning mortality at profitable levels.

Research done by Smithfield Premium Genetics® and published by Dr. Justin Fix of North Carolina State University (Fix, et al 2010) examined the relationship between piglet birth weight and weaning weight at 3-4 weeks of age. Their results are shown in the table below:

Table 2: Effect of birth-weight category and parity on mean weaning weight in a maternal line of barrows and gilts*

Birth-weight category	No. of piglets (N = 2467)†	Average parity of dam	Birth weight (kg)			Weaning weight (kg)‡	SEM
			Minimum	Maximum	Mean		
1	42	2.4	0.66	0.94	0.86	4.15	0.13
2	135	2.6	0.95	1.10	1.03	4.65	0.07
3	243	2.9	1.11	1.26	1.19	5.03	0.05
4	387	2.7	1.27	1.42	1.35	5.38	0.04
5	491	2.8	1.43	1.58	1.51	5.76	0.04
6	467	3.2	1.59	1.74	1.67	6.08	0.04
7	319	3.4	1.75	1.90	1.82	6.39	0.05
8	238	3.7	1.91	2.06	1.97	6.64	0.05
9	145	4.0	2.07	2.85	2.24	7.15	0.07

* Pigs were weaned either at an average of 15 days of age (pigs weaned at 14, 15, or 16 days) or at an average of 20 days of age (pigs weaned at 19, 20, or 21 days). Individual piglet birth weights were partitioned into nine categories which incrementally increased or decreased by 0.5 SD (0.16 kg) from the birth-weight mean (1.57 kg).

† Includes only pigs that survived until weaning.

‡ All weights differ from each other ($P < .001$; ANOVA)

They weighed more than 2400 piglets at birth, assigned them to birth weight classes, and then followed their weight at weaning. A linear relationship was found between piglet birth weight class and piglet weaning weight. Most noticeable was the weaning weights of less than 5 kg for piglets with birth weights less than 1 kg on average.

The authors also followed the pigs from weaning to market and tracked the cost and return of each piglet. They measured the piglets post-weaning mortality, growth rate and feed conversion, and eventual return when marketed. These results are shown in table below:

Table 6.3. Differences in predicted revenue ($) at harvest for pigs of various birth weights, compared to an average birth weight pig (1.4 kg)[1].

	Live Market Hog Price						
Birth weight, kg	$43.00	$45.00	$47.00	$49.00	$51.00	$53.00	$55.00
0.8	-$39.42	-$41.25	-$43.09	-$44.92	-$46.75	-$48.59	-$50.42
1.0	-$26.76	-$28.00	-$29.25	-$30.49	-$31.74	-$32.98	-$34.22
1.2	-$13.09	-$13.70	-$14.31	-$14.92	-$15.52	-$16.13	-$16.74
1.4	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00
1.6	$11.38	$11.91	$12.44	$12.97	$13.50	$14.03	$14.56
1.8	$20.60	$21.56	$22.52	$23.48	$24.44	$25.39	$26.35
2.0	$27.72	$29.01	$30.30	$31.59	$32.88	$34.17	$35.46
2.2	$33.03	$34.56	$36.10	$37.63	$39.17	$40.71	$42.24
2.4	$36.84	$38.56	$40.27	$41.99	$43.70	$45.41	$47.13

Based on these results, using input costs from the USA in 2010, it is clear that as the birth weight of the piglet increased, the profit of the pig at market weight also increased significantly.

The results from Fix et al (2010) can be used to develop regression effects of piglet birth weight on economically important traits such as pre-weaning mortality, post-weaning mortality, market weight, and the percentage of 'Full Market Value Pigs'. The results showed that as pig birth weight got below 1.4kg the chance of profit got less probable. Their research suggested that for each decrease in piglet birth weight of 0.1kg (below 1.4kg) the pre-weaning mortality increased by 3%, the post-weaning mortality increased by 2%, market weight decreased by 1.63 kg or 3.6 lb, and the probability of it being a 'Full Market Value' pig decreased by 2%. They defined a 'Full Market Value' pig as one that weighed more than 200 lbs (90kg), had no injuries, ruptures or other defects, and received full market value at the slaughterhouse. A pig that was not 'Full Market Value' received a discounted market price of 75%.

Using the above research based information, we can estimate the profitability of each birth weight class (1.4kg, 1.3kg, 1.2kg, 1.1kg, 1.0kg) using the PigProfitTracker® software. This software uses farm specific cost of production inputs, and user inputted performance and price information. A market carcass price of $84/cwt was assumed for FMV pigs, and $63/cwt for non-FMV pigs. A feed cost of $0.12 was used.

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The table below shows the relative performance and estimated profit for each birth weight class of piglet:

Variable	1.4kg BW	1.3kg BW	1.2kg BW	1.1kg BW	1.0kg BW
Pre-wean mortality	8%	11%	14%	17%	20%
Post-weaning mortality	5%	7%	9%	11%	13%
Percent 'Full Market Value' pigs	95%	93%	91%	89%	87%
Percent 'Pre-market' pigs	5%	7%	9%	11%	13%
Avg market weight (FMV pigs)	250.0	246.4	242.8	239.2	235.6
Profit (loss) for FMV pigs	15.00	12.26	9.48	6.66	3.79
Profit (loss) for non-FMV pigs	(-32.23)	(-33.82)	(-35.44)	(-37.11)	(-38.83)
Net Profit (loss) per pig marketed	12.64	9.03	5.44	1.85	(-1.75)

Based on the this information, as a piglets birth weight declines from 1.4kg down to 1.0kg, the average profit per pig also declines. And based on the information and assumptions in this table, piglets with a birth weight of 1.0kg or less will not produce a profit for the pig farmer. How does this then impact the genetic supplier? Over the past 10 years the genetic merit for litter size has increased greatly; however, the number of piglets with low birth weights has also increased as the litter size has exceeded uterine capacity of the sow. How should the genetic supplier account for this increase in low birth weight pigs in their genetic improvement program? A logical solution is to only give born alive credit to those pigs that have an adequate birth weight to be profitable. Therefore, the move towards selection for litter size born alive with birth weight above 1kg is recommended for use in improving profitable litter size in pigs.

Economic Impact of Sow Culling Rate and Sow Mortality Rate:

When a sow goes out of service from the breeding herd, either by voluntary culling or dying, there is a cost to the farm. Even if the farm is raising their own replacement gilts, these events incur a cost to the farm. The traditional comparison when making a decision to cull a sow has been to compare the performance of a 'potential cull' sow to that of a replacement gilt. For instance, if the performance of the older sow is 10.5 born alive and 80% farrowing rate; and, the performance of the parity 1 replacement gilt is expected to be 11 born alive and 88% farrowing rate, most herds have chosen to cull the older sow.

However, there is another factor to consider in making this decision: the performance and profitability of progeny from the parity 1 sow compared to those progeny from parity 2+ sows. Klobasa et al (1986) reported on differences in immunoglobulin synthesis in first versus multi-parity sows. Deen (2002) reported that gilt progeny are more likely die in the nursery than sow progeny. Morales et al (2006) and Burkey et al (2008) showed an effect of sow parity on piglet health status. And Miller (2008) and Miller et al (2012) examined the poor growth and survival of the progeny of gilts. Research presented at the Leman Conference has shown that progeny from parity 1 sows have a lower weaning weight, higher mortality, lower growth rate and higher medical costs. The magnitude of this difference is shown in the following table:

So now when the producer considers voluntarily culling a sow, they should take into consideration the salvage value of the sow, the cost of the replacement female, the reproductive performance of the replacement female, and the reduced performance of the progeny from the first parity sow compared to the progeny from the mature sow.

Item	Parity 1 Progeny	Parity 2+ Progeny
Weaning weight, lbs	11.7	12.5
Nursery mortality %	3.2	2.6
Nursery ADG (lbs/day)	0.90	0.96
Nursery medical costs	$1.79	$0.71
Finishing mortality %	4.31	2.95
Finishing ADG	1.62	1.69
Finishing medical costs	$1.52	$0.84
Estimated profit difference	-$5 to -$9 per pig	

There is a cost to both sow mortality and sow culling rate. If we consider the factors mentioned above, assume no difference in reproduction, use a $5 per pig reduced performance from gilt litters, and use the PigProfitTracker© software, the economic impact in a 2400 sow farm for a 1% increase in sow mortality is to lower profits by approximately $6057 per year. And the economic impact of a 1% increase in sow culling rate is to lower profits by approximately $1652 per year. When one considers how easy it is to see culling rate change by 5% or more, this economic impact becomes very significant.

Economic Impact of Post-weaning Mortality and Percent Full Market Value Pigs:

Within a commercial pork production system the size of the Grow-Finish farms is set by the sow farm size and pig flow. The pig farmer needs the correct number of nursery and finisher spaces (or wean-finisher spaces) to match pig flow from the sow farm. The role of these farms is to start the pigs on feed after weaning (the bigger the pig the better), then to sell the pigs when they reach the desired market weight. As a result, the growth rate, feed conversion and mortality of the pigs are very important traits. The return when the pigs are marketed are based on the number of pigs marketed with adequate size, as the packer will discount any pigs that are under a minimum market weight. There can also be some impact on price based on the meat quality of the pigs, but this is not usually a large impact.

So at the Grow-Finish farms, the fixed costs are based on the per head capacity of the farm, and variable costs are based on several factors for each pig, especially feed conversion and mortality rate and weight when mortality occurs. The return is influenced by the number of full value pigs marketed, the growth rate, and mortality rate. One important fact that bears mention here is that the pig's genetics for post-weaning traits comes equally from the sire and dam of the piglet.

The economic value of post-weaning mortality is well recognized. When the pig is weaned, then moved to the grow-finish facilities, it is expected to eat feed, convert the feed to lean gain, and then be marketed as a full value market hog. When a pig dies after weaning it has incurred much of the cost of a market hog, but gives no value in return. Using the PigProfitTracker© software and an assumption that the average weight of post-weaning mortalities is 100 lbs, the economic impact of a 1% increase in post-weaning mortality is $30,914 for a 2400 sow breed to finish farm.

The economic impact of a pig failing to meet the 'Full Market Value' standards is not as well recognized. In this case the pig does not die, but also does not reach market weight of at least 200 lbs. When this occurs the packer discounts the price given to the pig by at least 25%. Using the PigProfitTracker© software the economic impact of a 1% increase in non-Full Market Value pigs is $28,682. While these pigs do generate some return, they are very inefficient in their growth and the reduction in market return is quite severe.

Current Popular Genetic Marketing Strategy:

Several genetic suppliers have focused heavily on improving litter size and pigs weaned per sow per year, in both their genetic improvement programs and in their marketing strategies. Many customers who have switched to these genetic suppliers have seen correlated responses in higher sow mortality, higher sow culling rates, higher post-weaning mortality, and an increase in the percent of non-Full Market Value Pigs. What is the economic 'trade off' in this situation?

Using the PigProfitTracker© software, with a starting assumption of a 2400 sow breed to finish herd, and an economic assumption of $6/pig profit. Reproductive and performance starting points were 28 PSY, 45% culling rate, 6.2% sow mortality rate, 5% post-weaning mortality rate, and 4% of pigs were non-Full Market Value pigs as defined earlier in this paper. We then imposed three different scenarios with an increase of reproductive performance from 28 PSY to 31 PSY. In Case 1 (no correlated loss of fitness) we kept the sow culling and mortality rates, and pig mortality and non-FMV rates the same. In Case 2, we assumed a small correlated decrease in fitness traits, and in Case 3, a moderate decrease in the fitness traits. These results are summarized in the table below:

	Average	Case 1	Case 2	Case 3
PSY	28	31	31	31
Sow culling %	45	45	48	50
Sow mortality%	6.2	6.2	8	10
PW mortality %	5	5	6	7
% Non-FMW pigs	4	4	5	6
Profit	$382,560	$471,535	$382,058	$295,070

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If we just increase PSY from 28 to 31 and assume no loss in the fitness traits, then profit from the farm increases by 23%, as shown in Case 1. However, if we assume a slight loss of fitness as shown in Case 2, the annual profit from the farm is essentially the same as our 28 PSY starting point. And if we assume a moderate loss of fitness as shown in Case 3, the annual profit from the farm is reduced by 30% from our 28 PSY starting point. It is not logical to assume we can increase PSY from 28 to 31 with no loss of fitness in the sows or pigs, so Case 1 would not be an expected outcome. The lesson here is that the producer needs to consider both the increase in reproduction from the 31 PSY sow genetics, but also the producer should consider what level of loss in the fitness traits for the sows and pigs would be expected under their farm management and environment.

At this point, let me summarize several of the 'Take Home Points' from this discussion:

- Over the past 30 years the use of genetic technologies has made significant genetic improvement in reproduction, growth rate, feed conversion, and lean meat percentage.
- At the same time we have seen an increase in the percent and number of low birth weight pigs, which eventually have lower weaning weights, poorer survival, slower growth and less profit potential.
- Associated with this movement towards more extreme phenotypes we have seen a correlated reduction in the fitness traits of both sows and piglets (mortality rates, culling rates, sub-standard weaners and finishers)
- It is important that genetic suppliers include phenotypic structure in their genetic improvement programs as a way to minimize any loss of fitness.
- It is also possible for genetic suppliers to include fitness traits such as mortality rates and culling rates in their genetic improvement program, most likely through progeny test programs.
- For the commercial pork producer it is logical to focus their genetic program towards one that will maximize long term profits at their farm rather than chase short term improvements in targeted traits of current interest.

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“What is next in PRRS vaccination: Pursuit of broad heterologous protection”

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A presentation prepared for the 24th International Pig Veterinary Society Congress and 8th European Symposium of Porcine Health Management at the Royal Dublin Society (RDS) Dublin, Ireland 8th June 2016.
Porcine reproductive and respiratory Syndrome Virus (PRRSV) is the most economically significant infectious disease of swine worldwide. Due to the great impact of PRRS in many key swine producing areas of Asia, Europe and the Americas, the epidemiological situation created by this infection has become very complex. Perhaps an accurate picture of the current worldwide situation of PRRSV had been already anticipated in a conference offered in 2008 by Dr Jeffrey Zimmerman (Iowa State University) during that year’s London Swine Conference (London, Ontario, Canada) where it was said : “This period (last decade) has been marked by 1) A growing recognition of the high cost of PRRS to swine producers; 2) Continued producer frustration with the poor control of PRRS; 3) Heightened interest in regional elimination of PRRSV, but reluctance to proceed without more reliable methods of achieving the objective; 4) Reports (and “counter reports”) of newly emerging, highly virulent, PRRSV isolates; and 5) Innovation in the application of diagnostics to surveillance”.

Certainly the challenge in the PRRSV arena resides on the development of the tools that are necessary to warrant a technologically sustainable eradication of PRRSV. The research community can currently be described as “tri-fold split” in relation to which priorities should be assigned in order to achieve such technological sustainability: 1) Many would emphasize the idea of immediate eradication with strict management of biosecurity, with very meager and frustrating experiences as in many areas of dense endemicity and swine intensive production it becomes almost impossible to maintain large herds free of PRRSV infection 2) others are cautious about that previous approach and bring the fact that no major endemic infectious disease, human or animal, has been eradicated without the help of an efficient tool such an effective viral vaccine. Such tool, as needed for the case of PRRSV, is not here yet, but active research towards such goal is ongoing in America, Europe and Asia. We plan to update this IPVS audience on the efforts along that line. 3) Finally an additional group of researchers seem to be ready to argue that characterization of genetic control of resistance to disease and recent advances in transgenic pig studies may provide the solution to the PRRSV situation. Such view will be also presented by other speakers at this meeting today.

A major research goal of our laboratories at the University of Nebraska is the development of a new generation of PRRSV differential marker vaccines that would confer broad protection. Such goal is based on a major premise: the conviction that the use of vaccines will always be a cost-efficient method and the preferred approach to control PRRSV infections. As early as June 2007, a nationwide colloquium held by US PRRSV experts at the University of Illinois (Urbana-Champaign) (7) came to the conclusion that a new generation vaccine for PRRSV may require at least 10 years to reach the market (an accurate time prediction still valid today(2016) !), and that several technical approaches may be followed to develop such novel vaccine. However, the expert group concluded that the live, replicating type of vaccine seems generally to be the most favored, based on the more robust immunity that can develop in the pig after the application of this type of vaccine. This presentation will summarize the different avenues being explored for possibly improving the broadness of the coverage provided by current PRRSV vaccines. To improve the current vaccines it is essential to understand the basis of protective immunity generated by wt PRRSV upon natural infection.

What is currently known about PRRSV protective immunity

Our current knowledge on the basic mechanisms for PRRSV protective immunity is fragmentary. A significant degree of genetic diversity amongst the PRRSV strains circulating in the field exists. Likewise, a clear definition of what is meant by effective heterologous protection amongst PRRSV strains is still lacking. We have studied the resolution of persistence of wt PRRSV in convalescent animals. Contrary to other known examples of persistent RNA viruses, the persistence established by PRRSV is finite and seems to involve a low level of productive infection which progressively

Keynote Speakers

declines (i.e smoldering infection) until complete viral clearance takes place. During viral persistence, extensive modulation of the innate and acquired immune response takes place, although finally a firm convalescent immunity gets established and PRRSV is eliminated from the infected pig, although this cure sometimes takes up to 5+ months(1).

Using reverse genetics we have investigated the major modulating effects exerted by PRRSV on immunity and the main viral structures involved in that process.

A major immediate effect on the innate response upon PRRSV infection is a severe inhibition of 2 pro-inflammatory cytokines: IFN type I and tumor necrosis factor alpha. Such subversion of the pig's innate immune response seems to be primarily (although not exclusively) mediated by the two subunits of the nonstructural protein 1 (PRRSV NSP1 alpha and beta)(3, 17).

Regarding acquired immunity, perhaps the most compelling example of PRRSV modulation of the immunity is given by the aberrant timing of appearance of PRRSV-neutralizing antibodies. The PRRSV-neutralizing antibodies, that we have shown are a major correlate of protection, are produced by the pig very late in the infection process (at around 6 weeks p.i.). (11)The mechanisms of modulation of the acquired protective immune response by PRRSV may involve different strategies of immune evasion, including the display of decoy epitopes in the proximity of neutralizing epitope of the major envelope glycoprotein GP5(15), as well as glycan shielding of at least two envelope glycoproteins, GP5(2) and GP3(21).

To complete the puzzle, there still remains to ascertain the contribution towards protective immunity of other components of PRRSV. However, given the significant strain diversity of PRRSV, the major challenge for improvement of current vaccines consists of finding the basis for broadening their protective response,

What is being done to improve PRRSV vaccines, New knowledge on PRRSV biology, new PRRSV technology and PRRSV effective vaccination.....Are we there yet?

It is well accepted by now that the establishment of an efficacious immunologic memory specific against PRRSV will require mechanisms of antigen presentation that would mimic those used by the wild-type live PRRSV itself. In that respect the main avenues followed by different laboratories seeking the development of new generation vaccines X PRRSV are varied , although centered around two principal, alternative principles: 1) use of viral vectors that would replicate in vivo and would present PRRSV antigens to the immune system just like live PRRSV does. This progressive approach still awaits for more clear information on the complete set of immunogenic structural subunits that should be required for the establishment of an effective protective immunity, 2) the use of reverse genetic technology (infectious clones) for the improvement of the current MLV vaccines or the de novo development of live vaccines that would be rationally attenuated and endowed with a capacity to differentiate infected from vaccinated animals (i.e. fulfilling DIVA principles).

Above and beyond of the method(s) that eventually prove(s) to be successful in reaching effective homologous protection, a formidable additional challenge in all cases is represented by the need of defining the number of valencies or specificities that should be used in the formulation of the novel vaccines in order to ensure broad protection against PRRSV infection. The major research investment in the area of PRRSV in the US has certainly been on vaccine research and development. Although the current modified live vaccines may confer a solid protection when the infection or challenge of vaccinated animals takes place with a PRRSV strain of antigenic make-up similar to the vaccine strain, the protection against heterologous strains (i.e.: more distant from the vaccine) remains less than desirable. On the other hand, authentic evidence for any measurable level of protection obtained with inactivated (killed) vaccines remains to be obtained. Many experts also express doubts that PRRSV control and eventual elimination could be achieved without broadly protective vaccines that reduce shedding and transmission. Although more than 25 years have elapsed since the discovery of PRRSV, much remains unknown, as mentioned above, about the immunology of this virus. In particular, the exact nature of the protective immune response remains unclear and the goal of broadly protective vaccines for PRRS has not been achieved, due in great part to the highly variable nature of PRRSV, as well as the significant modulating effect that PRRSV infection seems to inflict on the host's immune response. Development of adaptive immune responses after infection of naive pigs with PRRSV or vaccination is known to be anomalous, with IFN

gamma-secreting cells appearing late and evolving erratically during the first weeks after infection(11, 12). Appearance of neutralizing antibodies is also delayed. Neutralizing antibodies may protect against infection if present in the body in sufficient quantities before infection, but they are not effective at clearing PRRSV during natural infections. PRRSV is able to modulate innate responses, probably through the regulation of IFN-alpha, other pro-inflammatory cytokines, and perhaps, IL-10 . While the mechanisms of protective immunity against PRRSV may not be fully elucidated, research from our own laboratory as well as others clearly indicate that PRRSV-neutralizing antibodies are important contributors to PRRSV protective immunity(10, 14). However, while it is clear that antibody may confer complete immunity to PRRSV, experiments indicate that other mechanisms, such as innate and/or cell mediated immunity, may also be protective (25). Further complicating immune protection against PRRSV is the pronounced diversity of this virus (13). PRRSV exists as two major genotypes, European (Type 1) and North American (Type 2). There is only limited immunologic cross-protection between isolates with these genotypes. Moreover, considerable variation exists between field isolates of each of these genotypes indicating continuing divergence of viral genomic sequences. The genetic diversity within each genotype is so elevated that allows a vaccinated animal to be re-infected by a different strain of the same genotype, a circumstance which further confounds our understanding of the host -PRRSV interaction. Sequence divergence has been shown to occur on serial passage between pigs, within persistently infected pigs and in pigs from infected farms. Furthermore, it is possible that different PRRSV strains are able to influence the immune system in different ways, which adds significantly to the confusion prevailing in this field(6).

Control of PRRSV has proven difficult, even with vaccination and protection against PRRSV infection remains a matter of primary importance for swine producers. Current PRRSV vaccines include 2 main types of products: modified live and inactivated virus adjuvanted vaccines, with the occasional use (mainly in the US) of inactivated autogenous vaccines made from indigenous field isolates. In June 2007, a meeting was held at the University of Illinois, College of Veterinary Medicine to discuss the state of current knowledge about PRRS vaccination(7). The meeting was attended by invited experts in PRRS, virology, immunology and vaccinology, as well as industry and major swine veterinarians. Major conclusions of this colloquium were that successful vaccination against PRRSV can be achieved and improved, with current MLV vaccines as the gold standard by which improvement is defined. The group of experts, however, advised that a final product would feasibly not available in at least 10 years and that three major technical challenges or knowledge gaps need to be overcome before such successful development of an effective new vaccine takes place. These are: 1) to identify structural components of PRRSV and host mechanisms involved in PRRSV protective immunity, 2) to understand the mechanisms involved in PRRSV attenuation in order to reduce virulence and/or increase immune responses of the vaccine strains and 3) to improve the meager protective efficacy of current vaccines against heterologous PRRSV strains, mainly by ascertaining what defines a heterologous strain in terms of protective immunity. Multiple evidences indicate that the best protection that can be obtained when vaccinating pigs is through the use of replicating, attenuated live vaccines(25). Inactivated vaccines, as we said above, appear to be ineffective(25). In addition, some immunogenic PRRSV structural subunits are being tested in different laboratories worldwide. Attention focuses specifically on the GP5/M hetero-dimer cloned in vectored vaccines. The GP5/M subunit vaccines produced are so far able to confer a relative level of protection, insufficiently protective and lower to that attained with live vaccines. This indicates that more needs to be known about the immunogenic role of other PRRSV structural components. Work in our laboratories at the University of Nebraska (4) points towards the important notion that GP2 and GP4, two minor glycoproteins located on the surface envelope of PRRSV and that have been the target of little investigation so far, are the viral components that interact with the virus receptor of the host cell Such role would make GP2 and GP4 central to the induction of a protective/neutralizing host response. These subunits, therefore should be included in the list of immunogenic components of PRRSV that would contribute significantly to protective immunity against PRRSV infection. It appears that there is a significant cell-mediated immune protection that can only be conferred, so far, by live vaccines, thus the inclination expressed by the expert groups at the Illinois symposium, towards the live vaccines, which in some cases have been already designed, through reverse genetics, as differential (DIVA) marker vaccines. Independently of the platform and technology to be used for the design of new generation PRRSV vaccines, it is clear that a major challenge yet to be overcome in PRRSV vaccinology should be that of circumventing strain diversity so to obtain a widely protective immunogen as stated in the conclusions of the Illinois PRRSV Vaccine Colloquium. Modified live PRRSV vaccines can confer homologous protective immunity that is considered to be close to or completely sterilizing immunity. On the other hand, however, the extent and duration of protection against heterologous strains may be variable and dependent on antigenic relatedness of the virus strain

Keynote Speakers

used for inoculation and challenge. Percentages of heterologous protection attained by MLV vaccines in pregnant sows have been described to vary widely: ranging between 52 % and 85.9 %. The wide range of heterologous protection empirically obtained by commercial vaccines dramatically exemplifies our current difficulties at precisely defining a heterologous level of protection in PRRSV.

Can we group the PRRSV strains (and so define valencies to be contained in vaccine) based on their antigenic rather than their genetic phenotype?

Phylogenetic analysis of hypervariable structural genes of PRRSV such as GP5 has been used extensively to group and study relationships between strains. Besides the GP5-based classification, no other antigenic grouping of the constellation of strains of PRRSV has been attempted until recently. Research now being conducted at the universities of Nebraska and Complutense of Madrid involves attempts to use cross-neutralization to systematically group PRRSV isolates by some important phenotypic trait such as the strain's antigenic make up. The notion that GP5 sequence alone is not a good predictor of the protective effectiveness of a vaccine strain and the realization that other possibly protective genes different than GP5 may induce neutralizing antibodies emphasize the need for better knowledge of other viral genes important for cross protection and broad antigenic coverage by vaccines. However, the level of knowledge at the level of the entire genome of PRRSV had been very scarce, with a limited number of strain genome-length sequenced until a few years ago, and many of these closely related sequences and/or lacking phenotypic data.

How to widen the cross protective effect of the current vaccines?

Different research teams are pursuing the goal of expansion of protective coverage of PRRSV vaccines following independent separate approaches. Overall, we can list at least three main lines of work that are directed at broadening the antigenic coverage of the PRRSV live vaccines:
In one case, the emphasis is being placed primarily in discovery, characterization and establishment of strategies to develop immunogens oriented at inducing broadly neutralizing antibodies that would be capable of inactivating antigenically diverse strains of PRRSV (16). The notion in place in this case is that the broadly neutralizing antibodies would be directed to conserved epitopes domains such as the contact residues that interact with the most significant cellular receptor for PRRSV (CD163), based on the CD163 interaction between the glycoproteins trimer as proposed by Das et al. (5), although recent solid evidence for the occurrence of broadly neutralizing antibodies may also occur in the matrix envelope of PRRSV(20)

Two other innovative approaches recently used to explore the construction of broadly protective PRRSV immunogens that would circumvent the heterologous variability of strains are based on bioinformatics analysis of the PRRSV genetic variability. Both of these approaches seek the design of immunogens that respond to the notion of centralized antigens that provide broad protection as originally described for human immuno-deficiency virus (8, 9). In one case (Virginia Tech University) the application of this technology to the PRRSV model has centered in using one or more structural glycoproteins designed as antigenic mosaics (19, 23, 24). Alternatively, another recent application of the centralized mosaic principle to on the design of a PRRSV live replicating immunogen (University of Nebraska) has been based on transferring over the concept of a single structural centralized antigen to the entire PRRSV genome, which can be virtually be considered as multiple centralized antigens of PRRSV(i.e including all structural and all nonstructural genes) coded for throughout the entire PRRSV genome(22).

XJ Meng' s lab (Virginia Tech campus) has extensively studied the adoption of the mosaic vaccine concept to the PRRSV model by means of molecular breeding through DNA shuffling, a process that would essentially mimic natural recombination but that forces it to happen at a much accelerated speed in a cell culture plate rather than in vivo. These authors developed initially a mosaic of PRRSV GP3 and PRRSV GP4 and M, while more recently they reported a mosaic containing all the structural genes shuffled for 6 heterologous strains cloned inside a backbone of a commercial vaccine, thus obtaining a replicating construct that they tested to be used as an experimental vaccine. While single shuffled GP DNA vaccine was useful to show some moderate cross protection as expressed by some sober cross-neutralizing titers, when the vaccine was formulated as a mosaic composed of all the structural genes of six donor heterologous strains , then exhibited some level of cross protection against heterologous strains challenge, although just a partial one.

On the other hand, Vu et al (University of Nebraska) used a set of 60 non-redundant, full-genome sequences of type-II PRRSV to generate the consensus genome (PRRSV-CON) by aligning the 60 PRRSV full-genome sequences, followed by selecting the most common nucleotide found at each position of the alignment. The resulting PRRSV-CON has the highest degree of sequence identity to the PRRSV field-isolates when compared to any current PRRS vaccine strains, both at the full-genome level and the individual gene level. These authors then synthesized an entire PRRSV-CON genome and pursued classical reverse genetics to generate PRRSV-CON cDNA clone which proved to be fully infectious and virulent when the PRRSV was recovered by classical reverse genetics. Cross protection trials were conducted using groups of recently weaned pigs assessing, viremia, average weight gain, viral load in tissues, and lung pathology against distant (widely heterologous) challenge strains. Remarkably, primary infection of pigs with PRRSV-CON virus conferred significantly broader protection than the prototype PRRSV strain FL12 when tested upon subsequent independent challenge trials with two unrelated widely heterologous PRRSV strains.

Summary/ Conclusions/what is next?

Bio-informatics analysis of PRRSV genomes may provide the clue for broadening the antigenic coverage of PRRSV vaccines and immunogens. DNA shuffling offers an opportunity for rational design of PRRSV MLV vaccines that possibly confer heterologous protection at the level of single structural genes or in the format of mosaics of multiple shuffled structural genes. In addition, shuffled PRRSV chimeric antigens, when targeted through DC-SIGN directly to DCs, elicited antigen-specific T cell immunity in pigs (18).

The synthetic PRRSV-CON virus can serve as the parental strain for the development of a novel PRRS live vaccine with broader cross-protection. The PRRSV-CON virus confers exceptional cross-protection against divergent PRRSV strains thus serving, as the current gold standard for PRRSV cross protection between heterologous strains. The PRRSV-CON virus can serve, upon adequate and complete attenuation, as a potential seed strain for the formulation of a broadly protective live attenuated PRRS vaccine. In addition, the occurrence of an exceptionally board protection like the PRRSV-CON virus provides an extraordinary reference tool to ascertain the mechanisms and correlates implicit in heterologous protection against divergent PRRSV strains.

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Keynote Speakers

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Genetic resistance - an alternative for controlling PRRS?

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Genetic resistance as a first choice of prophylaxis?

Breeding for disease-resistant pigs might be the ultima ratio in combatting infectious diseases. Regardless of whether pigs would be resistant sensu stricto, (i.e., the absolute prevention of an infection, or just tolerating the infection) minimal amplification and shedding of the pathogen and minimal effects on health and performance could be achieved. Thus, the infectious pressure in and between herds could be efficiently reduced, followed by diminished disease incidence, improved performance and product quality, reduced antibiotic treatment, improved consumer protection and increased animal welfare (Reiner, 2009).

Genetic resistance in the field

Disease-resistant breeds or populations are of considerable importance to livestock. A prime example is resistance to gastrointestinal nematodes in sheep (e.g., Stear and Wakelin, 1998). In pigs, however, examples of genetic resistance in commercial breeding programmes are sparse. Two examples are resistance to fimbriated F18 Escherichia coli (Vögeli et al., 1997), which causes post-weaning diarrhoea and oedema disease, and resistance to fimbriated F4 E. coli, which causes neonatal diarrhoea (Jorgensen et al., 2003). In spite of the currently limited commercial applications in swine, a wide range of genetic variation has been observed in genetic resistance to different bacterial, viral and parasitic diseases. A comprehensive search would identify differences in susceptibility/resistance in any host-pathogen relationship (Reiner, 2009). However, most of this genetic variation cannot be used, because of the difficulty in recognising favourable and unfavourable gene variants within the breeders. Their identification is impeded by highly variable and influential farm-specific environmental effects (e.g., pathogen load, immunity, housing, feeding and management conditions), the polygenic inheritance mode of most resistance traits, the limited availability of animal models and limited detailed knowledge of pathogenesis for most porcine diseases.

Improving genetic disease resistance

While classical breeding is generally inappropriate for efficient improvement of genetic resistance, evolved knowledge of the porcine genome combined with new tools and technologies – developed in the context of genome projects – have created new opportunities to dissect the genetic control of complex traits, including host responses to infection. As an alternative to classical breeding, responsible gene variants can be identified via experiments in selected populations that vary significantly in resistance/susceptibility, under standardised environmental conditions, including time point of challenge and quantity of the pathogen. Once the responsible gene variants are identified, breeders can be selected via marker-assisted and genomic selection. Provided there is societal consent, desirable gene variants can even be introduced into breeding populations via genetic engineering. In addition, understanding the genetic control of genetic resistance in molecular terms will improve knowledge about the underlying mechanisms of disease and disease resistance, thus promoting new and enhanced developments in diagnostics, therapy and prophylaxis.

Examples on the way

We have seen there are limited examples of applicable gene variants already in the field to improve genetic resistance in swine. However, the search for significant and applicable gene variants has developed into an ever-expanding and successful branch of clinical research, including viral (Pseudorabiesvirus [Reiner et al., 2002]; Influenza A [e.g. Yin et al., 2015]; bacterial Haemophilus parasuis [Wilkinson et al., 2010]; Actinobacillus pleuropneumoniae [Reiner et al., 2014a,b]; Mycoplasma hyopneumoniae [e.g., Shimazu et al., 2014]; Streptococcus suis [Wu et al., 2015] and parasitic diseases such as Sarcocystis [Reiner et al., 2007] and Ascaris suum [Scallerup et al., 2012]). More than 2,300 quantitative trait loci (QTL) have been published for health parameters, among them 263 for resistance against a broad range of pathogens (http://www.animalgenome.org/QTLdb/). QTL are gene loci which participate in the control of quantitative distributed traits such as milk yield, growth performance and disease resistance. The most remarkable results have been seen in resistance to PRRSV.

Keynote Speakers

Natural genetic disease resistance against PRRS in swine breeds and populations

Halbur et al. (1998) provided initial indications of genetic differences in susceptibility/resistance of pigs against PRRS. Duroc pigs showed lower performance combined with an increased severity of lung lesions and antibody titres after infection with PRRSV than Meishan pigs. Clinical abortion rates have been found to be associated with IFN and influenced by sows' genetics (Lowe et al., 2005). A genetic background for differences in performance, severity of lesions, viral titres, infected macrophages and immunological parameters has also been described by Petry et al. (2005), Vincent et al. (2005, 2006), Doeschl-Wilson et al. (2009) and Reiner et al. (2010), although differences were often small and partially inconsistent over time. Lean lines (Duroc and Hampshire) have been found to be more susceptible than lines selected for higher reproductivity. Ait-Ali et al. (2007) reported on favourable macrophages in Landrace pigs and assumed the density and distribution of CD169 and IL-8 levels to be critical factors. High levels of IL-8 and low levels of IFN were also associated with PRRSV resistance by Petry et al. (2007).

PRRS resistance: tracking down causes by genome-wide genetic association and differential expression studies

These results provided enough evidence for a genetic background of PRRS resistance and remarkable differences in susceptibility between breeds or at least populations. For the next step, pigs differing at most in susceptibility/ resistance were used in experiments to take a detailed look at their genetic peculiarities. Three major setups were applied initially: QTL analysis (Haley and Andersson, 1997) and genome-wide association study (GWAS) were used to identify chromosomal areas (QTL study) or single nucleotide polymorphisms (SNPs) associated with PRRS phenotypes (e.g., degree of viremia, lung lesions and performance after PRRSV infection (Boddicker et al., 2012, 2013, 2014)), and differential expression experiments to detect genes via differences in their expression levels in susceptible and resistant pigs (Schroyen et al., 2015). The most significant results have been achieved by Joan Lunney (USDA), Bob Rowland (Kansas State University) and colleagues, particularly in the context of the PRRS Host Genetics Consortium (PHGC, for a review, see Lunney et al., 2016).
Based on up to 60,000 SNP markers together with new statistical tools, more than 30 QTLs for resistance against PRRS have been mapped to 11 chromosomes http://www.animalgenome.org/QTLdb/ (Boddicker et al., 2012, 2013). As part of the PRRS Host Genetics Consortium, a genome-wide association study based on 190 pigs from a commercial breeding line and the Illumina PorcineSNP60 BeadChip detected associations with viral load and body weight after PRRSV infection. A major QTL region was mapped to chromosome 4 (SSC4), explaining 16% of genetic variance for virus load with a frequency for the favourable allele of 0.16 and a heritability of 0.30 (Boddicker et al., 2012).

GBP5 as an important candidate gene for PRRS resistance

The highest linkage disequilibrium was found for SNP WUR10000125. The interferon-induced guanylate-binding protein 5 gene (GBP5) was identified as the most likely candidate in a total of eight consecutive and independent trials (Boddicker et al., 2012, 2013, 2014). This gene was differentially expressed and validated in different pig populations (Koltes et al., 2015) and an intronic SNP (rs340943904) (close to WUR10000125, but not on the 60k SNP chip) was found to be responsible for introducing a splicing site that truncated the C-terminal 88 amino acids in the recessive A-allele. GBP5 is involved in immune response to bacterial and viral infection in different species, namely in the inflammatory response and the assembly of the inflammasome, which strongly depends on the C-terminal 67 amino acids which are highly conserved between species. Although the exact role of GBP5 in PRRSV defence remains to be identified, this SNP is the putative quantitative trait nucleotide (QTN) (i.e., the SNP most likely to be responsible for the QTL on SSC4). In addition, Boddicker et al. (2014) only found small effects for resistance to PRRS on SSC1, 5, 7 and X. Further research is needed to show the generality of these findings in other global pig breeds.

A second approach to detect underlying molecular differences in PRRS susceptibility/resistance was performed via microarray analysis (e.g., Schroyen et al., 2015). Several immune response pathways were upregulated after infection and several hundreds of differentially expressed genes were detected, but this did not lead to a simple identification of directly responsible genes. One major concern with differential expression (DE) studies is that many differentially expressed genes (A) do not necessarily need to carry the responsible mutation. Instead, their differential expression is achieved via the products of other genes (B) binding to the promoter and 5´UTR region of the A genes. These B genes,

however, do not necessarily need to be differentially expressed, provided the relevant mutation leads to an amino acid exchange, resulting in altered efficiency of the gene products of genes B at the promoters of genes A. Thus, they may not be detected in DE studies. Thus, the strength of DE studies lies mainly in the detection of the gene networks and pathways involved.

Further candidate genes and the role of genetic variability and autochthonous breeds

Type I interferons are a heterogeneous group of cytokines, important in antiviral response. Genetic variation has been linked to susceptibility to viral diseases, and PRRSV has been found to suppress type I IFN production as a major strategy for evading the immune system. Sang et al. (2011) discovered more than 100 polymorphisms in 39 functional genes from the type I interferon family. More than 20 polymorphic mutants have been linked with differing anti-PRRSV activities in vitro (Sang et al., 2011).
Rare breeds, often autochthonous to some regions or countries and poorly adapted to modern pig production, are a valuable source of rare gene variants with sometimes unexpected effects. Rare or even lost SNPs might be (re-) introduced via gene editing methods. However, this requires knowledge of these effects and, therefore, the breeds carrying the rare SNPs. One potential example was provided by Li et al. (2015) who identified an Mx1 (myxovirus resistance protein 1) promoter variation, potentially associated with PRRS resistance. Mx1 exhibits potent anti-RNA viral activity and is involved in early host defence against PRRSV (e.g., Chung et al., 2004). A second candidate gene, potentially involved in PRRSV resistance, with the valuable allele preferentially restricted to Chinese autochthone breeds, is the ubiquitin-specific protease 18 (USP18; Li et al., 2014).

Most genes and molecules involved in PRRS pathogenesis escape detection via genetic and genomic methods, if they are not variable in sequence or expression, or if this variability is not present in the studied populations. Thus, it is extremely important to add fundamental virus research to the pathway for resolving pathogenesis and resistance. The most comprehensive input here has come from Hans Nauwynck and his group at Ghent University. Once detected, pigs from different breeds and populations can then be screened for variation in these basic genes.

Support from fundamental virus research: the PRRSV receptors

At least six cellular molecules have been described so far as putative receptors for PRRSV, including CD163, the cysteine-rich scavenger receptor (SRCR; Calvert et al., 2007), sialoadhesin (CD169; siglec-1; Duan et al., 1997), CD151 (Wu et al., 2014), heparin sulfate (Jusa et al., 1997), vimentin (Kim et al., 2006) and CD209 (Huang et al., 2009). These have been reviewed by Zhang and Yoo (2015).

CD163

CD163 is restrictively expressed in cells from the monocyte/macrophage lineage, and significant expression is exclusively found in activated (major) tissue macrophages, together with complement and Fc receptors, other scavenger receptors, and receptors for mediators, adhesion molecules and growth factors (Van den Heuvel et al., 1999). Macrophages not or only newly involved in inflammation and defence do not express CD163 to any substantial degree (Backe et al., 1991). Activation of TLRs (e.g., TLR 4 by LPS or other pathogen-associated molecular patterns (PAMPs) increases IL10 (Weaver et al., 2007), one of the strongest upregulators of CD163 in humans (Williams et al., 2002). A second important activator of CD163 is stress (glucocorticoids) (Högger et al., 1998; Van den Heuvel et al., 1999).
One major function of CD163 is in the receptor-mediated internalisation of pathogens, and coincidentally its role as an innate immune sensor for Gram-positive and Gram-negative bacteria, linking bacterial infection with inflammation (e.g., via pro-inflammatory cytokines like TNF (Van Gorp et al., 2010)). While ligands are delivered to early endosomes, CD163 recycles to the plasma membrane for new rounds of endocytosis (Schaer et al., 2006). However, some pathogens have developed mechanisms to evade these physiological processes and use the receptor to enter their host cells, namely African swine fever virus (ASFV; Sanchez-Torrez et al., 2003) and PRRSV (Calvert et al., 2007; Van Gorp et al., 2008; Patton et al., 2009).

Keynote Speakers

CD163 and PRRSV

Several attachment factors have been studied extensively as potential PRRSV receptors, with CD163 and CD169 identified as the most likely candidates. However, only CD163 has been shown capable of conferring PRRSV permissiveness to cell lines unsusceptible to PRRSV, even in the absence of CD169 (e.g., Calvert et al., 2007; Van Gorp et al., 2008; Patton et al., 2009; Van Gorp et al., 2010). It was shown that PRRSV permissivity was conferred by CD163 independent of the PRRSV genotype involved (I [EU] or II [US]) (Calvert et al., 2007; Lee et al., 2010). The role of CD163 was finally proven in the gene editing experiments of Prather et al. (2013) and Whitworth et al. (2015), who transferred PRRSV resistance to pigs by deleting CD163 sequences from the pigs' genome. However, they had no success when deleting CD169.

Not all cells that express CD163 can be infected by PRRSV, which is important for achieving PRRSV-specific cell tropism. In addition, PRRSV shows a restricted tropism for subsets of porcine macrophages in vivo. These two observations might be due to CD163 quantity or interaction with other co-receptors (Zhang and Yoo, 2015). The expression of CD163 on macrophages in different microenvironments in vivo may determine the replication efficiency and subsequent virulence of PRRSV (Patton et al., 2009).
Minor differences between experiments in terms of efficiency of PRRSV replication seem to be more a matter of receptor interaction and membrane lipid environment than of differences between PRRSV genotype, although variability of the pathogen itself also affects the quantitative outcome of PRRSV replication.

CD163 domains

CD163 consists of nine cysteine-rich tandem repeats, forming the extracellular scavenger receptor, a transmembrane domain and the intracellular cytoplasmic tail. PRRSV entry seems to be related to domain 5, the two PST domains and a few others, but not with the complete receptor (Van Gorp et al., 2010). The interacting PRRSV glycoproteins responsible for receptor binding and infection are GP2a, GP3, GP4 and E, especially GP4 and GP2a. Replacing ORFs 2a to 4 with EAV ORFs keeps the virus viable and infectious, but protects macrophages from infection (Tian et al., 2012). Glycosylation of GP2a and GP4 by glycans can have different effects on PRRSV replication, depending on the PRRSV genotype (Wissink et al., 2004). However, transients are floating, because of the role of lipids and cholesterol from the lipid rafts of the outer plasma membrane that interact with embedded proteins and receptors (Yang et al., 2015).

Supporting receptors

Sialoadhesin(CD169) is a transmembrane glycoprotein, a lectin, restricted to activated tissue macrophages and involved in cell-cell interaction. Expression can be induced in macrophages by IFN and IFN during the inflammatory process (Rempel et al., 2008). The receptor facilitates pathogen interactions and uptake of sialylated pathogens (e.g., HIV (Rempel et al., 2008) and PRRSV (Duan et al., 1997; Delputte et al., 2007)). Sialoadhesin seems to facilitate attachment of PRRSV, eventually together with heparin sulfate, and internalisation, but not replication of the virus (Van Gorp et al., 2008, 2010). A gene editing experiment that deleted CD169 found full PRRSV-permissive macrophages and unaltered viremia and antibody production in the pigs (Prather et al., 2013). The authors conclude that sialoadhesin is not required for PRRSV infection and that the absence of the CD169 gene neither prevents PRRS nor alters PRRS pathogenesis.
Heparin sulfate is widely distributed on the surface of most mammalian cells. Heparin sulfate, heparin-like proteins and proteoglycans bind to GP5/M heterodimers and the M complex of PRRSV in a virus-dependent manner (Jusa et al., 1997; Delputte et al., 2002). Together with sialoadhesin, heparin sulfate seems to propagate the interaction between PRRSV and its specific receptor(s), but heparin sulfate is not necessarily required for PRRSV entry (Delputte et al., 2002).
CD151 is involved in numerous cell functions and cell signalling. Silencing the gene made susceptible cells resistant, while overexpression made resistant cells susceptible to PRRSV, making CD151 a key receptor for PRRSV infection. Blocking CD151 by microRNA (miR506) prevents the cells from being infected (Wu et al., 2014). However, CD151 is restricted to the erythroid cell lineage and is not expressed on macrophages.
Vimentin and CD209 are further putative receptors that might be involved in varying efficiency of PRRSV binding and replication (Kim et al., 2006; Huang et al., 2009).

A gene editing breakthrough?

All these results regarding PRRSV receptors finally led to gene editing experiments and the knockout of PRRSV-receptor function in CD169 (Prather et al., 2013) and CD163 (Whitworth et al., 2015) in gene-edited pigs. Loss of CD169 did not affect PRRSV replication, but gene-edited pigs without CD163-receptor function were protected from PRRSV. The pigs showed no fever, respiratory or other clinical signs, and no lung pathology, viremia or antibody response after inoculation with a NVSL 97-7895 PRRSV isolate in a controlled study. In addition, no problems occurred during pregnancy and growth of the piglets until challenged with the PRRSV isolate at the age of 3 weeks.

What is gene editing?

The goal of improving livestock genomes by direct manipulation is old. Its development was accompanied by serious problems in terms of site-specificity (precision), efficiency of the methods used and a lack of acceptance in wider society. Thus, unlike transgenic crops, no transgenic livestock has ever gained commercial approval (Laible et al., 2015). All these problems may have been overcome with the introduction of gene editing via CRISPR/CAS9 (Doudna and Charpentier, 2014). The system combines an endonuclease with a specific short guiding (sg) RNA sequence. Like a primer in PCR, this sequence provides accurate specificity, while the linked enzyme can cleave and modify the DNA at exactly the position targeted by the sgRNA sequence. The system can also be used in a multiplex manner to edit different genes in one step. However, comparable to the amplification of incorrect sequences by primer mismatching in PCR, care must be taken not to introduce unintended mutations anywhere in the genome at off-target sites. New methods have been developed to minimise the off-target size problem (Mali et al., 2013). Originally, the CRSISPR Cas9 system was part of natural, sequence-specific immunity in bacteria, responsible for the introduction of DNA double-strand breaks into invading plasmids and phages (Garneau et al., 2010). Taken together, concerns about the precision and efficiency of transgenics have been overcome by this new method in previously inconceivable way.

Concerns about genetic resistance as a tool to combat PRRS

Gene editing and regulation by authorities?

Gene editing can introduce mutations to the genome without adding any footprints associated with the technology. Thus, genome modifications cannot be distinguished from natural mutations (Laible et al., 2015). Further, vectors to introduce foreign DNA into transgenic organisms, which might prove hazardous to consumers, are no longer needed. Both factors have led to the enthusiastic acceptance of gene editing by most researchers, the scientific community and industry. Unlike transgenic organisms, gene-edited plants and animals may not need regulatory oversight (Waltz, 2012; Lusser and Davies, 2013), provided the human germ line is not involved. Animals and products might not even be classified as genetically modified organisms (GMO). However, as the methodology explodes and a vast number of gene-edited livestock will be produced in the coming years, societal interpretation is currently difficult to predict. However, restrictions are likely.

Patented PRRS resistance?

A second concern is related to upcoming patents – not for the PRRS-resistant gene-edited pigs, because their creation calls for the protection of this intellectual achievement. However, what about patents that will cover the complete CD163 or others in their role of preventing PRRSV replication? Society must decide whether naturally occurring gene variants with a potential to improve health and welfare should exclusively be exploited by individual companies. The future always brings changes and the ability of populations and species to change is based on their genetic variability. As any individual can carry a maximum of two alleles at any position in the genome, resource populations often lose rare alleles with decreasing population size. These alleles, once lost, cannot be reintroduced by gene editing, as their favourable effects have never been documented. A single breed is not enough to fulfil the different demands of diversified markets worldwide.

Keynote Speakers

Side-effects of CD163 knockout?

The facts outlined above for CD163 show that this protein has not evolved solely as a PRRSV receptor, but with a broad spectrum of tasks, including the elimination of pathogens other than PRRSV and the regulation of the immune system. CD163 awaits the discovery and evaluation of further involvements and mechanisms. Any knockout of CD163 as a whole or in part needs meticulous investigation of impacted pigs under field conditions, including the effects of other pathogens and adverse conditions. Work is currently in progress and results are expected in future.

Sustainability of genetic resistance?

Will CD163 knockout protect against other and upcoming PRRSV strains? One common concern surrounding disease resistance is whether pathogens will be able to adapt to host resistance like they acquire resistance to antibiotics. Acquiring resistance is possible in theory, but the method is unlikely to be similar, because there are no plasmids harbouring information for an arbitrary switch to new tropism. Some examples of single mutations provoked tissue or even species shift under “natural” conditions, although species shifts are very rare events in the evolution of most viruses (Forsberg et al., 2001). A prime example is the Influenza A virus (e.g. Mänz et al., 2013). Other examples arise from the Coronaviridae (e.g., SARS [Feng, 2005]) and TGE/PRCV (Ballesteros et al., 1997) viruses.
The specific risks of mutations altering cell or species tropism include high mutation rates in RNA viruses and conditions that lead to the crowding of different pathogens or strains and high infectious pressure. These conditions are found in PRRSV-infected pig herds. The strong “make or break” for PRRSV replication due to the presence or absence of CD163, which could provide a unique and widespread solution to the PRRS problem, runs a strong risk of being overcome by one or few SNPs. On the other hand, differences in oligo- or polygenic pathways that are involved in the immune answer to PRRSV infection are much more complex. This complexity hinders elucidation and the “make or break” principle of resistance. However, if such natural resistance could be implemented, the odds that PRRSV would overcome these genetic changes would decrease. However, it is impossible to predict exactly what will happen.

Conclusion

The detection and knockout of CD163 as the receptor responsible for PRRSV replication in pigs is a milestone in modern pig production. Complete or even partial elimination of PRRSV replication will lead to a significant improvement in the disastrous situation in infected herds, with significant impact on welfare, production efficiency, performance and consumer protection. The complete function of the receptor and its reasonable modification still requires elucidation, and the evaluation of other gene variants involved in immunological pathways is just beginning. Thus, the coming years will see redoubled efforts to transfer this new knowledge and new possibilities to the herd level. The success in using genetic resistance as an alternative in controlling PRRS will not only be measured by the effect on microbiological and health parameters, but also on the availability of the newly acquired PRRSV resistance in pig populations worldwide.

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Keynote Speakers

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Keynote Speakers

The usefulness of vaccines to control the spread of pathogens in pig populations

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Introduction: background on population effect of vaccines

The most studied effects of vaccines used in pig production and more generally in animals are the direct effect in individuals. The main expected impact is related to a decrease in the clinical outcome of the disease related to the infection. In some cases, the decrease in pathogen shedding at the individual level is also documented for the vaccine. However, it is well known that the majority of the vaccines used in animals and humans can been referred as leaky vaccines because they only reduce without fully preventing the risk of infection [1]. All the vaccines used in pig production can be categorized as such.

The population effect of a vaccination programme is the result of a collective impact of individual vaccination on the transmission of infection in that population. While direct individual protection is the major focus of mass vaccination programmes (simultaneous vaccination of the whole population), the global population effects also contribute indirectly to individual protection through herd immunity. The population impact of vaccination programme depends on three main factors. First, it depends on the epidemiology of the pathogen, and more specifically on its transmission potential. This is most readily summarized by the basic reproduction number, denoted R_0. This is the average number of infectious individuals generated during the infectious period of a single typical infective in a large susceptible population. Second it depends on the impact of the vaccine on the ability of individuals to contribute to the transmission of the infection, which we could be summarized as vaccine efficacy against transmission. This combines both reduction in individual susceptibility through direct protection and the effect of the vaccine on the infectiousness of infected individuals, including any changes induced in the infectious period (amount of pathogen shed, duration of shedding). Third, population impact depends on the vaccination programme and in particular on the vaccine coverage in the population [2]. This latter is closely linked to the basic reproduction number specific to the pathogen. From a general point of view, and other things being equal, the larger the R_0) value, the harder it will be to eradicate the infection from the pig population.

In a "homogeneously mixed" population, eradication will be achieved if the proportion successfully immunized, p, is higher than a critical value $P_0=1-\frac{1}{R_0}$. To eradicate infection, the effective reproductive number denoted R must be brought below 1. It corresponds to the modification of the transmission of the pathogen because of the successful immunization of a proportion p of pigs within the population. Hence x=1-p, x being the remaining non-immune fraction, and $R=R_0x$ which should be less than 1 for eradication. From this relationship, the critical fraction of the population to be immunized is $P_0=1-\frac{1}{R_0}$. In consequence, the larger the R_0, the higher the coverage needed to eliminate the infection (figure 1). Under this theory, each vaccinated individual also confers some protection to the general population, since those they would have infected are now less likely to get the disease. This is known as herd immunity [3].

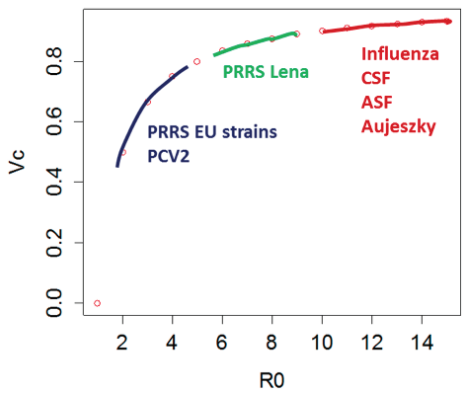

Figure 1: Evolution of the vaccine coverage (V_c) (proportion of the population to be immunized) according to R_0 value (specific case of a "perfect" vaccine).

One of the crucial applied factors to account for in the field is that vaccines are not perfect. Being injected with a vaccine does not always confer full immunity; in such cases the critical threshold applies to the proportion of the population that needs to be immunised (given immunity). Partial protection can arise in many ways: the vaccine can reduce pig's susceptibility to infection (lower the risk of becoming infected), it can reduce subsequent transmission if the pig becomes infected, or it may speed up recovery. Such vaccines are described as leaky. Again a critical vaccination threshold can be established. With R_v being the number of secondary cases produced by an infectious individual when the entire population is vaccinated, then the threshold becomes

$$V_c = \frac{R_0 - 1}{R_0 - R_v}$$

R_v needs therefore to be less than one, or else it is not possible to eradicate the infection. Figure 2 shows that when a vaccine is leaky and those vaccinated only have partial protection, then eradication is possible as long as $R_v < 1$, but the thinner the difference between R_0 and R_v, the higher the proportion of the population to be immunized.

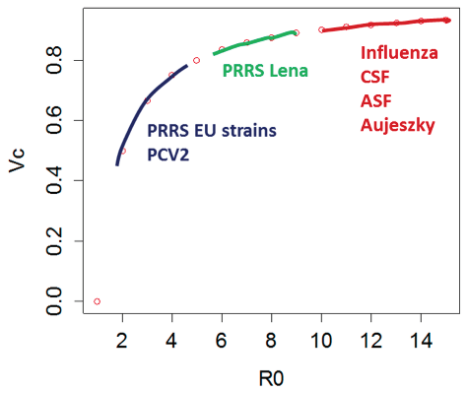

Figure 2: Evolution of the vaccine coverage (proportion of the population to be immunized) according to R_0 value and for different values of R_v.

Establishing control programmes using vaccine tools at the population scale requires therefore quantitative knowledge on the ability of vaccines to decrease transmission within the target population. The next section will provide information on (1) the available methodologies to estimate the reproduction number in vaccinated population; and (2) an overview of published data related to pig pathogens.

2. How to evaluate the effect of vaccines on transmission

When considering the use of a vaccine as an epidemiological tool to eradicate or at least decrease the impact of a disease in a population, it is crucial that the vaccine can prevent the spread of the pathogen. The classical studies based on a vaccination-challenge do not shed light on how transmission occurs, as they focus on characteristics of the infection that are important for the individual pig. Therefore, it should be studied how the pathogen is expected to spread within a vaccinated population and which factors may influence transmission. Qualitative studies describing transmission of pathogens to susceptible sentinel pigs do not address the question of (i) to what extent does the pathogen spread; and (ii) are the observed differences in transmission between vaccinated and non-vaccinated populations relevant and significant. To answer those pivotal questions, transmission has to be quantified. Data that can be used to quantify transmission can be obtained from laboratory experiments and from field observations. The specificity of these infectious processes is that the incidences of infection in different groups within the same population are dependant, generating non-linear infection dynamics. In this specific case, non-linear mathematical models are useful to generate hypotheses about how transmission occurs, to understand epidemiological processes, and to evaluate the possible effect of control measures [4].

Transmission experiments

The effectiveness of a vaccine to prevent transmission of virus should be determined in homogeneous groups, which implies that all the animals in one group should be vaccinated while all the animals in the control group remain unvaccinated. The animals within groups are assumed to be identical i.e. 'equally' infectious or susceptible, and to mingle freely within the group (equal probability of contact). Transmission cannot be estimated properly using heterogeneous groups (adding susceptible animals within a group of vaccinated-challenged pigs). Indeed, vaccination generally reduces both the infectivity and the susceptibility of animals. Infecting unvaccinated pigs in a group of vaccinated contact pigs might result in extended virus spread because the unvaccinated inoculated pigs are more

Keynote Speakers

infectious than vaccinated inoculated pigs would have been. In the other way round, unvaccinated contact pigs in a group of vaccinated inoculated pigs might also result in extended spread because of higher susceptibility. The principle of the statistical analysis of transmission experiments can rely on the final size of the observed epidemics [5]. A stochastic Susceptible Infectious Recovered (SIR) model is used to represent the different infection chains according to the initial number of susceptible and infectious pigs leading to a distribution of the final size of the epidemics. The advantage is that only the situation at the beginning and the end of the experiment is needed. However, when all the susceptible pigs get infected during the infectious process, the reproduction number tends to infinity and only the lower bound of the confidence interval can be estimated. More frequently, the incidence of infection in the contact pig population is used to estimate the transmission parameter, the duration of infectiousness and the reproduction number. Estimation can be based on the maximum likelihood method [6] or non-linear regression models [7]. These studies require a follow up of the individuals with time to get precise data on the number of new infections in the contact susceptible groups at each time intervals (between two samplings). The protocol (number of pigs to be inoculated, number of contacts, time-interval between two samplings) must be thought according to the available knowledge on the pathogen transmissibility.

Field studies

The limitation of transmission experiments is the possibility of extrapolating results to the field. Consequently, a field study in the target population is the ultimate test for a vaccine when possible. However those studies are often based on a follow-up of infection chains using a marker of the target pathogen infection. The use of serology is often the only solution affordable for large scale studies in farm populations but the antibodies markers of the infection have to be distinguished from vaccine-induced antibodies. This is only possible with the use of DIVA or marker vaccines such as those used for Aujeszky disease control. By studying infection chains (based on seroconversion against gE) in sow herds vaccinated every 4 months, only minor outbreaks of Aujeszky disease were observed despite the regular introduction of new susceptible animals in the herds with an estimated R in vaccinated herds of 0.7 [8]. Another field study compared the number of Aujeszky vaccinations: once (around 14 weeks of age) and twice (11 and 15 weeks of age) [9]. They used data on seroconversion against gE to estimate the reproduction number using the same approach as described for transmission experiments, i.e. a stochastic SIR model to represent the chains of infection leading to a distribution of the final size of the experiment (frequency of geE positive pigs at the end of the finishing period). Using those data they showed that the double vaccination reduced the reproduction number from 3.5 (single vaccination) to 1.5 (double vaccination) without leading to eradication. Some discrepancies with experimental results can lie in several factors linked to the status of the animals at vaccination time, sub-optimal vaccination, climate, other infections…

	Aujeszky disease	Classical Swine Fever	*Mycoplasma hyopneumoniae*	*Actinobacillus pleuropneumoniae*	PRRSv	Influenza	PCV2
Publications	[4, 5, 8-12]	[13-19]	[20, 21]	[22]	[23-25]	[26]	[27]
Number of publications	7	7	2	1	3	1	1
General effect	Significant reduction of transmission	Significant reduction of transmission	No significant reduction of transmission	Reduction of infectivity	Significant reduction of transmission	Significant reduction of transmission	Significant reduction of transmission
Consistency between published papers	Good for experimental studies	Good	Good	/	Good	/	/
Type of study	Experimental and field studies	Experimental studies only	Experimental and field studies	Experimental study	Experimental studies only	Experimental study	Experimental study
Remarks	Field studies indicate possible limitations	Variability due to vaccines, route of administration, delay vaccine-challenge		Separate evaluation of susceptibility and infectivity	Good results with vaccine and challenge strains of same genotype	Even with heterologous challenge strain	Experimental study, heterologous challenge strain

Table 1: Summary of some published quantitative data on the effect of vaccination on transmission of pig pathogens

3. Available data on the quantification of the impact of vaccination on transmission of pathogens in pig populations.

A summary of the available quantitative data on the evaluation of vaccine efficacy towards the reduction of pathogen transmission is given in Table 1. The most numerous studies have been carried out for Aujeszky disease and Classical Swine Fever because of the importance of this kind of information in order to eradicate those notifiable diseases with the help of vaccine tools. The majority of the studies are based on transmission experiments, except for Aujeszky disease for which several field evaluations were also carried out. There is generally a good agreement between published papers showing either the significant ability of available vaccines to decrease transmission (with sometimes an expected reproduction number below 1 in experimental conditions, e.g. Aujeszky disease, CSF, PRRS) or sometimes a clear absence of effect on transmission (Mycoplasma vaccination). The available data are very useful for incorporation within mathematical models designed to assist decision makers [28]. However, differences highlighted between experimental evaluations and field studies suggested possible mitigation of effects due to more complex interactions between risk factors such as the viral strain, the heterogeneity of contacts within populations, co-infections or rearing practices. Further work is therefore needed to investigate the effect of vaccines on transmission in co-infection situations, especially because several targeted pathogens are rather involved in syndromes such as the respiratory disease complex than in pure monofactorial diseases.

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Keynote Speakers

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Earning money from pigs – what do we need to consider from the wider food system?

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Abstract

The paper describes the theoretical underpinnings of economic analysis of the pig system and the origin and significance of the commonly used rules of thumb such as piglets per sow and feed conversion ratio. It also looks at the relationship between the production losses caused by animal disease and the costs of managing such problems. This relationship can be affected by animal health investments in research, education and infrastructure, which can only be done by larger societal organisations such as pig producer groups, large companies and governments. Getting the balance on these core fixed cost investment is the basis of good pig health. Yet a focus of the pig sector – the pig food system – cannot only look at the farm level production of the pig. It needs to take into account that the pig sector has evolved and is now global in its reach. Feed is brought from around the world for the pigs and the pork and pig products generated are processed, retailed and consumed in a global market. These changes have been relatively recent and have generated positive aspects of greater pork availability at relatively cheap prices. Yet there are other aspects that cause concern such as disease management and emergence of new health problems, competition for feed resource leading to input price uncertainties and the power of people and organisations in the pig sector generating downward pressures on pig prices. These wider aspects of the pig sector need careful consideration if the sector is to manage pig health and welfare in effective ways. The paper discusses these aspects and makes suggests that pig health is everyone's responsibility with a need for One Health approaches.

Key words: Pig production; economic analysis, animal health, food system

Introduction

The use of economic tools for farm level management and the incorporation of impacts of animal health are well covered in the literature (see Chapter 7 of Rushton, 2009; the review by Marsh, 2009). These pieces of work go through a standard process of needing to have systems in place that collect the appropriate data on production parameters, inputs and outputs and the markets that critical such as feed and liveweight prices. The data are combined to provide information in the form of gross margins and enterprise budgets in order to give an impression of the profitability of an individual pig enterprise such as fattening. Where changes of a system are required there is an array of tools available which are all based on the basic principle of comparing additional costs versus the additional benefits. These include partial budget analysis for changes that have a short time frame to cost benefit or investment analysis where changes occur over a period of years and there is a need to assess the time value of money. Where aspects of the changes are uncertain decision trees provide a useful means to examine the sequence of events within the decision making process and to add probability to prices or technical issues that uncertain and need to be quantified to give an understanding of the risks involved.

The process described has great merits in guiding pig producers and others working across the pig sector in decision making. Yet many choose not to develop systems of data collection, capture and analysis to provide evidence to inform the decision making process, instead people are drawn to rules of thumb or heuristics to guide their actions (Gilbert and Rushton, forthcoming).

This paper therefore sets out to provide a theoretical background as to why the economic assessment tools add value and then to raise issues outside the farm that need to be understood and at times challenged to ensure that pigs are kept in healthy and welfare friendly conditions and that pig farmers are given a reasonable chance of making a sustainable living from their endeavours. The paper will cover the theoretical basis of the tools and then discuss the wider pig sector – pig food system. It will identify some of the major changes that have occurred in the recent past and how these have impacted on the viability of the pig farmers. It will conclude with future challenges and how these will

Keynote Speakers

need to be researched in order to educate the current and future people in the sector in order to ensure it continues to provide sustainable livelihoods and a safe and stable food supply.

Theoretical basis of economic analysis

The tools described briefly in the introduction have a theoretical basis which contributes to the overall message of economics which is the allocation of resources with competing demands in order to achieve the greatest possible efficiency. This efficiency is often constrained by the level of technology available which for the pig system would include housing, genetics, feed and nutrition, animal health and overall management. The pig production system therefore include a good mix between hard technical knowledge and soft skills with regards to processes of managing staff to achieve levels of pig husbandry, health and biosecurity. In the case of animal health there is a range of possibilities in terms of understanding immunological development of the animal to the development of vaccines that provide lifelong protection from disease.

From an animal health perspective there are some critical concepts for the application of economics at the farm level which include production, productivity and profitability; expenditure-cost function and differences in cost structures. These are explained in more detail in the following sections.

Production, productivity and profitability

A farm level pig system will utilize a range of inputs such as feed, labour and housing in order to generate a range of outputs which could be gilts, weaner pigs from fattening, finished pigs for slaughter and cull sows and boars for slaughter. Some would also include that the pig system generates manure and effluent which is some situations is valuable either in the fertilization of land and/or the generation of biogas, yet in other situations can create unintended and therefore negative environmental impacts. A simple representation of this system is shown in Figure 1.

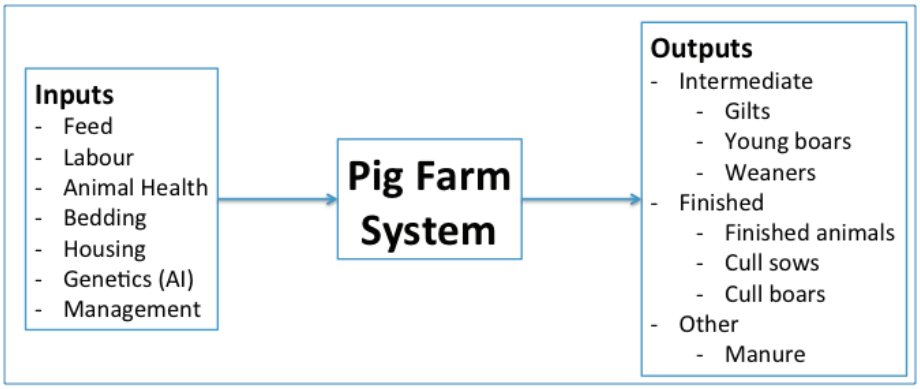


Figure 1. The pig farm system.

The representation of the system provides useful information in terms of the key inputs and outputs. Yet it lacks any quantification which is needed to make this an analytical structure. At the core there are data needed on the number of sows in the pig systems, the rates at which they are reproducing, the number of piglets per farrowing, the mortality rates of all animals. These population parameters are well known by farmers and veterinarians and are often reduced to heuristics such as number of piglets per sow per year. Overall if the data are available on the number of sows – the scale factor – and the number of piglets per sow per year – a productivity proxy – then it is possible to estimate the production of the system, which is the total number of finished pigs per year. This appears relatively simple for a system that has breeding and fattening pigs with the main output being animals finished to a desired slaughter weight. The use of the sow as a denominator for the productivity measure reflects the investment in this animal for the system as a whole, she not only has a significant monetary value, her presence also indicates that the farm has invested in building, facilities and labour to manage her adequately.

Where the pig system is focused on fattening animals for slaughter the focus will be on the number of animals, the growth rates, mortality, the target liveweight and the amount of feed used. These data are sufficient to estimate the total number of animals produced in a set time period – the production and the productivity of the system through calculating the feed conversion ratio (FCR). The productivity proxy focuses on the critical input – feed and the critical output – liveweight. FCR is therefore another very useful heuristic for the pig farmer.

The piglets per sow and FCR work because they have the same unit of measure for the numerator and denominator, live animals and kilogrammes, respectively. However where to generate the overall productivity of a pig system requires inputs and outputs to be converted into a common unit of measure and this is frequently a monetary value.

Figure 2. Pig system productivity.

The same data can be used to estimate the profitability of the pig system by simply calculating the difference between the value of the outputs and the value of the inputs. Economic farm management tools such as gross margins and enterprise budgets are a representation of the profitability of the system.

$$\text{Pig farm system } \textbf{productivity} = \frac{\text{Monetary Value of Outputs per Unit of time}}{\text{Monetary Value of Inputs per Unit of time}}$$

The overall use of these three measures of a pig system are important in their management. Production needs to be estimated in order to provide a basis of the value of the output and to make sense of whether it is useful to the people owning and running the business there is the need to combine this production estimate with key inputs. The two basic rules of thumb used in the pig industry, piglets per sow and FCR, simplify data collection, capture and analysis yet do not capture all the information needed for a business looking to improved productivity and profitability. To achieve the latter the business needs data across the range of inputs and outputs and the prices of these resources. Understanding the technical efficiencies of the system and the market prices becomes a basic tenet of managing the pig system and attempting to achieve outcomes that are both viable for the pig producers and sustainable for society. The wider market issues are returned to when thinking about the pig system as a whole.

Animal health – production loss - cost function

With the pig system decisions are made on the allocation of resources for animal health. At one extreme a disease or health problem could be ignored and the farmer accepts a level of loss. At the other a disease may not be tolerated and the farmer spends money on technologies and management systems to avoid the disease being present on the farm. Other diseases may have a combination of acceptable presence and hence a loss with some systems of management and investment to ensure they do not become too problematic. This is a verbal description of the animal health production loss-cost function that John McInerney and colleagues such as Keith Howe described back in the 1980s (McInerney et al, 1992). Figure 3 provides a graphical representation of this relationship.

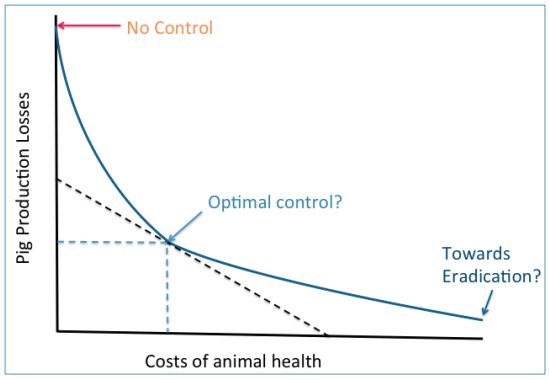


Figure 3. Animal health production loss-cost function (modified from McInerney, 1996).

The optimal point indicated relates to the relative price of the production loss and the control measure (black dotted line in Figure 3). Where pig prices are high and pork highly valued in society with technologies for disease management cheap the slope of the black dotted line will reduce and the point at which the slope of the loss-cost line meets this cost line will be further to the right, indicating the greater inventive to reduce disease. However, if disease management technologies are expensive relative to the price of pigs the line will be steeper and the disease control incentives will move towards the no control scenario. The important issue for animal health professionals to recognize is that the relative price of pigs to disease control measures will have an influence on the levels of disease control management levels employed by farmers. From an economic perspective understanding the markets for pigs and for animal health technologies becomes a critical issue to gain insights of farmer incentives to management disease and the subsequent pig disease burdens that a population of pig producers are willing to accept. The ability to influence these markets therefore becomes an important aspect of pig health policy.

Keynote Speakers

This theoretical work also provides a very useful basis for examining the impact of animal disease as it covers both the losses in production and the costs of control. This model has been followed in the estimate of disease impact in the UK (Bennett, 2003; Bennett and Ijeplaar, 2005) and more recently in Australia (Lane et al, 2015). These national studies provide a basis to rank diseases in terms of the overall importance of impact, and they also indicate where there is an imbalance between the costs of disease management versus of the losses in production. Further work has been done at global level by the World Bank (2011) which focuses only on the losses of animals due to disease. This study, while a good initiative, is flawed by the lack of resources available in collecting farm-level data on disease occurrence. There is a growing need for a more comprehensive body of work on the impact of animal disease which should be updated in order to prioritise diseases and the resources used to manage animal health problems (Rushton and Gilbert, 2016).

When interpreting these theoretical frameworks and their application to real pig farm situations it is worth remembering that points along the line represent a mean and there will be a range around this line. The loss-cost line itself represents a technical frontier, and is an indication of what is currently known about a disease or health problem and what technical solutions are available to manage the problem. Investments in research and education can therefore alter both the shape and the direction of the frontier, a technology shift. From the perspective of the individual pig farmer they will rarely have the economic means to influence the shape of the technical frontier, to shift this frontier requires either a group of pig producers to work together and invest in research and education and/or for a larger societal group such a local, national or international public organisations to act to support the industry. In the more recent times some of these major funding issues have been fulfilled by the large private philanthropic charities.

Costs – fixed and variable

Not all costs are equal some will relate directly to an animal disease management process and could be defined as variable costs. Others cannot so easily be assigned to animal disease management as they make use of the organisations involved in managing animal disease in society. They include investments in research and education and also critical infrastructure such as animal health diagnostic laboratories. In addition, they include the investments in management and coordination of the animal health professionals and also the pig farmers themselves. These can be defined as fixed costs.

During his work on FMD in Thailand Professor Clem Tisdell looked at this issue more careful and proposed that countries who did not have an investment in fixed cost elements of their animal health system will find it difficult to incorporate and succeed with individual disease management campaigns. Tisdell (2009) developed a theoretical framework around his arguments. These require a modification of the loss-expenditure model, which recognizes that some investments underpin good animal health systems and these tend to be lumpy investments (see Figure 4).

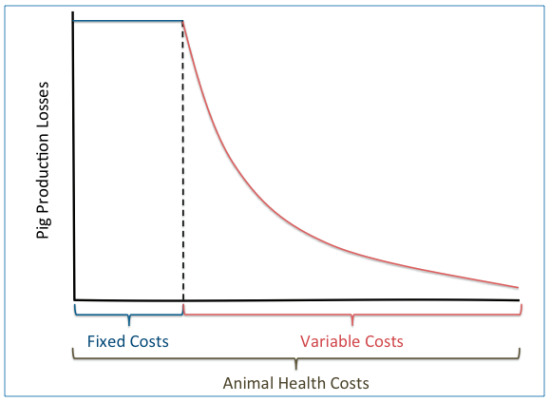


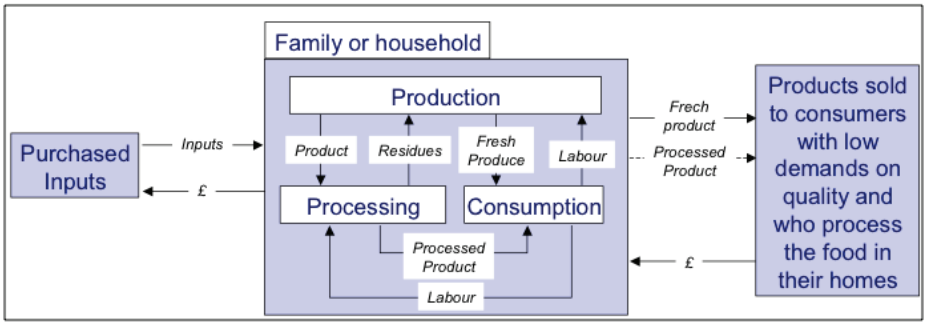
Figure 4. Loss-cost animal health function recognizing the need for fixed as well as variable costs.

The importance of this theoretical observation is that a successful pig sector will need to have investments in research, education and coordination in order to have levels of pig health status that is optimal for society as well as for the individual producer. The underlying investments require commitments and financial resources all those who benefit within the pig system from consumers to traders and retailers, the pig producers and the input suppliers.

The pig food system

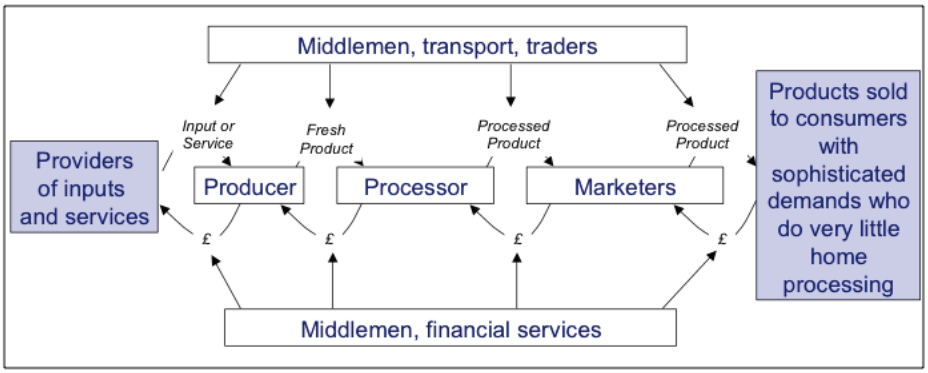
Over a relatively short the world has moved from relatively simple pig value chains that make up the overall pig food system to increasingly complex ones. In the simple pig value chains a high proportion of produce was either consumed in the household or in local and regional markets. The pig was also largely fed on resources local to the household either from their own holding or collected in the local environment. In addition, in many cases the pig was slaughtered either in the household or community and processed within the household (see Figure 5).

Figure 5. Simple livestock value chains (adapted from Rushton, 2009).



The simple pig value chains still exist around the world and in many parts of Africa, Asia and Latin America pigs in such systems can be found. However, increasingly the pig population and pork production comes from a more complex value chain. In the complex pig value chains, that are now dominant in many parts of the world, primary production has complex relationships with consumers through processing and marketing companies. The links in the chain are maintained by middle men, transport companies and finance groups. Where the value chains become integrated, i.e. owned and controlled by one company, the middlemen disappear. In addition, the consumer demands have become more sophisticated for processed food and food with zero risk of food-borne diseases (see Figure 6). These pig value chains are global with feed grains produced in one part of the world, shipped to pigs in another part and then the animal slaughtered and processed with consumption of product in different places.

Figure 6. A schematic diagram of the dominant complex pig value chains (adapted from Rushton, 2009).



The adoption of more complex pig value chains has not been gradual, rather it appears to have been in jumps. A large jump in the pig industry appears to have taken place in the 1950s and 1960s which brought together a series of technological advances in genetics, nutrition, animal health and general management of the chains. There is also an influence of the globalisation of pigs and pig product movement, associated with technological changes in transport and storage. Of greatest relevance were the investments of governments on pig nutrition and breeding at the start of this period along with significant resources and political commitment to animal health programmes. In the background was the green revolution which generated significant land productivity changes in grain and oilseed crops. These crop technology changes have made feed grains and oilseed cakes more accessible to producers with prices in relation to pig prices affordable to have production systems that are managed with concentrate feeds produced off-farm.

The period from the 1960s has seen an expansion in the numbers of pigs across the world and the level of pork available for consumers. This has been a combination of the greater scale of the industry and an improved productivity – particularly output per sow and increased output per unit of feed – during this period. The pork sector as a whole has managed to be so efficient that it has been able to produce more product at prices that relative to other goods in society have reduced. Consumers have therefore benefited from these changes.

Keynote Speakers

The world is now entering into a new anthropocene age reflecting the growing human population and the ability of people to modify the environment. Some of these impacts relate to increasing competition for land and the soils, water with an emphasis on potable water and air. The pig sector and the simple and complex pig value chains it contains plays a major role in this new age as it remains the major source of meat for human consumption and a major user of feed and oil seed crops that occupy a significant land area. In addition to these land use changes, there has been much evidence of changing patterns of diseases in the pig sector. Some relate to the emergence of new diseases such as Nipah virus in Asia (Epstein et al., 2006) and concerns of the association of human incidence of Japanese encephalitis (van den Hurk et al. 2008; Solomon, 2006). In addition the emergence of the highly pathogen H1N1 (Zimmer & Burke, 2009) created both a massive response and impacts on human health. In the background have been changing patterns of diseases that affect only pigs such as PMWS (Alarcon et al, 2013), PRRS (Nathues et al. forthcoming) and more recently PED (Wang et al, 2014). Finally there are a growing concerns on the public health issues, initially with food borne diseases such as salmonella, but more recently with the need to manage antimicrobial use and the associated antimicrobial resistance (Rushton et al. 2014; van Boeckal et al, 2015).

Conclusions - Present and future challenges

Current pig systems are very reliant on very complex arrangement of input supply, slaughtering, processing and retailing. The primary production of the pig is therefore not independent and requires significant levels of organization of transport, financial and management that come from outside the farm area. From this very brief review of the economics of this system three present and future challenges are highlighted:

1. Price of feed grains and oilseed cakes which make up a significant component of the inputs of any pig system across the world regardless of whether this is for an intensive or extensive system.
2. Price of paid for the finished pig. Information across the food system has to be of such quality that there is a transfer of prices in recognition of the costs of production and return to investment and management of the pig production unit.
3. Changing animal health issues and support for animal health investments with particular issues around:
    a. The use of antimicrobials and the need for research on the systems that are less reliant on AMU and more aware of AMR
    b. Emergence of new diseases that cause major economic impacts and the re-emergence of PRRS
    c. Introduction of new diseases such as ASF into the European system

The first of these challenges relate to the increasing competition for land and water for crops, something not under the control of the pig sector, yet something that the pig sector has an effect on. It is possible that future pig systems will need to have a change of feed inputs and therefore the existing ways in which pigs are produced may well need some further thought. The price of the finished pig has been wrestled from the producer and is in the hands of people who manage the pig food system closest to the consumer. Pig producers need an awareness of how to manage this power shift to ensure that they are given prices that adequately reflect their investments in production, health and welfare for the animal. For a reflective review of losing power the Bowman et al (2012) provide an excellent analysis of the UK pig sector and the problems created by lax government policy, weak producer organization and powerful retail interests.

The final challenge requires a much more powerful societal examination of the investments required in a One Health context. Pig health is everyone's business even if it only initially impacts on the pig. Pig diseases undermine productivity of the system and ultimately increase scarce resource use per unit of pork produced and pork prices for consumers. Pigs diseases that are zoonotic have much larger costs of management and greater economic impacts. The need for investments in research, education and infrastructure for pig health and production has never been greater and this investment needs to recognize that the pig sector is a complex systems with global reach.

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Keynote Speakers

Pig Welfare: where should we be by 2025?

Peter Stevenson, Compassion in World Farming

The question of where pig welfare should be by 2025 is intertwined with the broader question of what does a healthy future for the pig sector look like.

As regards welfare the EU pig sector needs to respond to the recognition by the EU Treaty that animals are "sentient beings" and that "full regard" must be paid to their welfare requirements. Globally, the pig sector should respect the OIE *Guiding principles for animal welfare*; these stress that "the use of animals carries with it an ethical responsibility to ensure the welfare of such animals to the greatest extent practicable".

Sow stalls

From this perspective sow stalls (also known as 'gestation crates') are unacceptable. EU pig farmers deserve to be congratulated as the vast majority are now in compliance with the EU ban on sow stalls. Moreover, the move away from sow stalls is gathering pace worldwide.

Sow stalls have also been prohibited in nine U.S. States[1] and in New Zealand.[2] The Code of Practice for Canada calls for a phase-out by 2024.[3] The Australian pork industry has committed to voluntarily phasing out sow stalls in favour of group housing by 2017.[4] All major pork processors in Brazil have announced they will phase out the use of sow stalls.[5,6,7] The South African Pork Producers organisation has committed to a phase out date of 2020.[8]

In 2007, Smithfield Foods, the world's largest pig producer, committed to phase out gestation crates by 2017 (although sows are housed individually until confirmed pregnant), and the company announced in 2016 it has moved over 80% of its pregnant sows from crates to group housing.[9]

The International Finance Corporation – which is part of the World Bank Group - has published a Good Practice Note *Improving Animal Welfare in Livestock Operations*.[10] This points out that "There is an international trend from sow stall use towards group housing systems, with or without limited stall use in the four-week period after mating".

I would hope that by 2025 the use of sow stalls will have come to an end worldwide.

Despite the EU ban on stalls, many EU sows continue to be confined in sow stalls and farrowing crates for over 20 weeks of the year. Regrettably, the EU ban permits the use of sow stalls during the first four weeks after service. This exception to the ban was granted due to concerns that mixing sows in early gestation may be detrimental to embryo development and survival. However, a number of studies have found that with good system design and skilled management mixing in the early stages of pregnancy need have no adverse effects on reproductive performance.[11,12] The first four weeks' exception to the ban should be brought to an end.

Farrowing crates

By 2025 the use of farrowing crates should have been replaced by free farrowing systems. A number of such systems are available and research shows that piglet mortalities in loose farrowing systems can as low as, or lower than, in crates.[13,14] Loose farrowing systems that have been developed include the Solari Pen, the 360° Freedom Farrower, the Danish SWAP pen and the free farrowing system developed in the UK by Scotland's Rural College and the Newcastle University (known as PigSAFE).[15]

Castration

Well before 2025 surgical castration should have ended. In 2011 stakeholders from throughout the European pork chain agreed to voluntarily end surgical castration by 2018. Progress, however, has been slow. Effective alternatives already exist. In some countries, including here in Ireland, male pigs are reared entire. If reared beyond the age of sexual maturity, entire males carry a higher risk of boar taint than castrates, but methods of detecting boar taint on the slaughter line are increasingly being developed and used.

The simplest option is immunocastration: boar taint vaccine. By stimulating the pig's immune system to temporarily delay puberty, the vaccine reduces not only boar taint, but also aggression and sexual behavior that can cause stress and injuries. It is unacceptable to put pigs through the pain of castration when a viable alternative is available. Fears of consumer rejection are holding back uptake of immunocastration. It is the responsibility of the industry and retailers to explain to consumers that this is not a hormone and that the meat is safe to eat. A substantial proportion of male pigs are immunocastrated in Australia and Brazil so it is clearly possible to achieve consumer acceptance.

Environmental enrichment and tail docking

The importance of activity for pigs has been established for many years. In the 1980s Stobla and Wood-Gush reported that in semi-natural conditions pigs spend 75% of their daylight hours in activity – rooting, grazing, exploring their world.[16] Recognising this, EU law has since 2003 required pigs to be given "permanent access to a sufficient quantity of material to enable proper investigation and manipulation activities, such as straw, hay, wood, sawdust, mushroom compost, peat".[17]

The European Food Safety Authority (EFSA) has concluded that enrichment materials should be complex, changeable and destructible[18] and that chains, plastic chewing sticks and balls are not effective enrichment materials.[19] Regrettably, most EU pig farmers ignore this law providing either no enrichment or just metal chains.

A Technical Report prepared for EFSA states that an "appropriate enrichment material can be defined as a material which stimulates exploratory behaviour for an extended length of time, preferably comparable to the level of occupation provided by straw." [20] The Report adds that "all new data reinforce the importance of providing suitable enrichment materials to allow expression of species relevant behaviours and reduce risk of injurious biting".

A European Commission Recommendation published in March 2016 provides that enrichment materials should be edible, chewable, investigable and manipulable. In addition the Recommendation states that the materials should be provided in such a way that they are "of sustainable interest, that is to say, they should encourage the exploratory behaviour of pigs and be regularly replaced and replenished".[21]

Guidance accompanying the Recommendation states that optimal materials include "straw, (from cereals and legumes), green fodder (hay, grass, silage, alfalfa, etc.), miscanthus pressed or chopped, root vegetables (e.g. turnips, fodder beet, swede) when used as bedding".[22] It adds that chains and hard plastic piping are of marginal interest and "should not be used as essential or single component of pig enrichment materials. They can provide distraction but should not be considered as fulfiling the essential needs of the pigs. Other materials should also be provided."

A closely related legal provision prohibits routine tail docking. It provides that farmers may only lawfully tail dock if they have first tried to prevent tail biting by "other measures" and in particular have changed "inadequate environmental conditions or management systems" but nonetheless still have a tail biting problem.

Scientific research helps us to understand which conditions are "inadequate" as it has identified the factors that are most likely to cause tail biting. EFSA has concluded that "the largest risk for being tail bitten is the lack of appropriate enrichment".[23] If there are no enrichment materials or only chains, toys or plastic objects, the farmer has failed to change "inadequate environmental conditions". Accordingly, the farmer has not fulfilled the conditions that would allow him to lawfully tail dock. Most EU pig farmers ignore this law and routinely tail dock without any attempt to provide meaningful enrichment.

Keynote Speakers

Provision of enrichment is not the only factor needed to prevent biting. Other elements that can trigger tail biting must also be addressed; these include correct balance of nutrients in feed, heat and cold stress, air quality, competition for food and space and poor health.[24] [25]

The Technical Report prepared for EFSA stresses:

"An intact curly tail may well be the single most important animal-based welfare indicator for weaned, growing and finishing pigs .... In addition, it stands for high-quality management and respect for the integrity of the pig."

Farmers who get their pigs through to slaughter age without either tail biting or docking will be operating a very good system.

EU pig farmers should now comply with the law as a matter of urgency. Worldwide farmers should provide effective enrichment and end routine tail docking well before 2025.

A more ambitious approach is needed as to what is meant by good welfare

Thinking about welfare and welfare science tend to focus on preventing poor welfare rather than on promoting positively good outcomes. This minimalist approach risks giving too narrow an ambit to what constitutes good welfare

Some areas that tend to be overlooked are brought to light by Lyall Watson in *The Whole Hog*:

"I know of no other animals that are more consistently curious, more willing to explore new experiences, more ready to meet the world with open-mouthed enthusiasm. Pigs are incurable optimists and get a big kick out of just being".

Fortunately, there is growing recognition of the need to take a less restricted view of what constitutes good welfare. This too will present fresh challenges to the pig sector. The Farm Animal Welfare Committee, an independent body that advises the UK Government, stresses that all farm animals should have 'a life worth living' and a growing number should have 'a good life'.[26] It insists that "each farm animal should have a life that is worth living to the animal itself, and not just to its human keeper". FAWC continues: "Achievement of a life worth living requires provision of an animal's needs and certain wants ... Wants are those resources that an animal may not need to survive or to avoid developing abnormal behaviour, but nevertheless improve its quality of life."

A new paper by Mellor stresses that it is necessary not only to minimise negative experiences but also "to provide the animals with opportunities to have positive experiences".[27] Such experiences can arise "when animals are kept with congenial others in spacious, stimulus-rich and safe environments which provide opportunities for them to engage in behaviours they find rewarding. These behaviours may include environment-focused exploration and food acquisition activities as well as animal-to-animal interactive activities, all of which can generate various forms of comfort, pleasure, interest, confidence and a sense of control."

The pig sector needs to adjust to a rapidly changing environment

The pig sector has much to do to respond to these expanded perceptions as to the scope of welfare. Moreover, the pig sector finds itself in a rapidly changing environment. Intensive farming is increasingly seen as contributing to antibiotic resistance, environmental degradation and poor animal welfare. High levels of meat consumption are recognised as unhealthy and as playing a key role in driving the world to dangerous levels of climate change. In the EU the pig industry lurches from one crisis to another. The pig sector will have to embrace fundamental changes if it is to survive.

Consumers are demanding welfare improvements. A new European Commission Eurobarometer survey reports that more than nine in ten EU citizens believe it is important to protect the welfare of farmed animals (94%) and 82% believe the welfare of farmed animals should be better protected than it is now.[28]

Intensive pig farming and the intensive production of the grain used as animal feed have led to water pollution[29], soil degradation[30] and biodiversity loss[31].

Public disquiet about the high use of antibiotics in intensive farming is growing. The pig sector is seen as contributing to the declining efficacy of certain antibiotics used to treat serious human disease. The *Review on Antimicrobial Resistance*, established by the UK Government, calls on the EU to reduce farm antibiotic consumption by around two-thirds.[32]

The *Lancet Infectious Diseases Commission* has called for" the development of health-orientated systems for rearing of animals" that are not dependent on regular preventive use of antibiotics.[33] Such systems would build improved health and immunity by reducing stress (e.g. by enabling animals to perform natural behaviours), avoiding overcrowding, minimising mixing and ending early weaning in pigs.[34] [35]

High levels of meat consumption are increasingly being questioned. Studies show that, on a business-as-usual basis, our diets alone - with their high levels of meat consumption - will by 2050 have taken us over the Paris Climate Agreement's target of limiting rises in global temperatures to well below 2°C.[36] [37]

Our consumption of high levels of red and processed meat - that have been made possible by industrial farming - can lead to obesity, diabetes and heart diseases.[38] [39] Most damaging of all, the pig sector's products – red and processed meat – have been classified as 'probably carcinogenic' and 'carcinogenic' respectively by the World Health Organisation.[40] As examination of the tobacco industry will show, any sector whose products are seen as carcinogenic faces an uphill struggle.

In March 2016 the EU agreed a new package of measures to support the struggling pig sector. These include storage schemes, promotion campaigns and a drive to find new export markets. At the heart of the crisis is overproduction. Announcing the new measures the President of the Agriculture Council stressed that "reduction of supply is necessary".[41]

The pig sector needs to move away from mass production of cheap commodity pigmeat to producing high quality meat but in lower quantities than at present. Moving to less but better meat in EU diets would benefit consumer health and the environment and would help mitigate climate change.

A switch to quality production could see farmers being properly rewarded for their work and skills provided that consumers were willing to pay fair prices for high quality pigmeat. Many consumers may well be willing to pay more if they are informed about the different modes of production and their implications for natural resources and pig welfare. In addition, pigmeat must be labelled as to farming method so that consumers can play a part in supporting a high quality pig sector.

At present industry promotional materials tend to give a misleading impression of the way in which pigs are reared. This is exemplified on the website for this conference which includes a photo of pigs with access to the outdoors which is extremely rare in EU pig farming. Because consumers are misled into thinking all is well they do not realise that there are problems which they can address by helping to drive the market for high welfare pigmeat.

Government too must play its part in helping the industry to restructure. The EU and the Member States must stop using EU funds to support industrial pig farming. At present CAP Pillar 2 funds are being used to subsidise industrial pig production for example by giving financial support for the building of industrial pig operations.[42] [43] Such funding should be stopped with the money that is saved being used to increase support for high quality pig producers. For example, the German state of Lower Saxony pays a premium of 16.50 per pig tail that is not docked or bitten when the pigs arrive at the slaughterhouse.[44]

Conclusion

The current industrial model of pig production cannot survive. The pig industry needs to reinvent itself; it needs to become a producer of high quality meat produced to good environmental and animal welfare standards. Routine preventive use of antibiotics must be replaced by health-orientated systems. The industry needs to understand that meat consumption may decline as studies show that the current thigh levels of consumption are unhealthy, environmentally damaging and make it most unlikely that we can meet the Paris target of limiting the rise in global temperatures to well below 2°C.

Keynote Speakers

From the welfare viewpoint, the industry should rapidly move away from all use of sow stalls and farrowing crates. Routine tail docking must be brought to an end and effective enrichment should be provided. In addition, selection for excessive litter sizes and routine teeth clipping should come to an end. The industry needs to respond to the developing awareness that welfare entails not only preventing negative states but also providing animals with opportunities to have positive experiences including pleasure and positive engagement with other animals and their environment all of which can generate a sense of well-being.

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Keynote Speakers

Antibiotic resistance; bad past and the bright future?

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The pig industry has made great strides in the past decades in terms of efficiency and cost effective productions systems. However with intensification has come increased challenges in the control of infectious diseases. Whilst improved genetics creates the potential for greater productivity, and programmed nutrition delivers on this potential, suboptimal animal health status will undermine any potential gains. The sector has to be mindful of maintaining consumer confidence in pork meat if it is to grow and prosper. Animal welfare issues undermine consumer confidence but also stressed animals are more susceptible to infectious disease necessitating antibiotics. The overuse of antibiotics has resulted in the selection of antibiotic resistant microbes, which creates both an animal and human health problem as a result of reduced therapeutic options but also draws adverse publicity on the sector with sensational headlines like “Superbugs in our pork” etc.” The response of some the major brands in the food service and retail sectors is to talk about seeking “antibiotic free production” and an expectation is being created that this is achievable. There is no doubt that antibiotics have been used as a substitute for good husbandry practices and this has contributed to the current situation. The use of antibiotics as in feed growth promoters has been banned in the US since January 2016 and has caused alarm amongst many US pig producers who fear their viability will be threatened but the ban introduced in the EU in 2006 only served to force producers to look at alternative approaches. However in the EU we are still left with the situation that overprescribing of antibiotics still exists in some parts of the sector. Poor biosecurity leading to an increased incidence of immune suppressing viral infections on some farms leads to secondary bacterial infections which require antibiotic treatment to limit mortality. Vaccination programmes, and alternative immune boosting initiatives, won’t deliver their full benefit where viral diseases are endemic therefore the biosecurity protocols must be robust.

The primary responsibility for prudent use of antibiotics in the pig industry lies with the small number of prescribers (specialist pig vets) and the end users, the pig farmers. Reduction in the use of antibiotics is required and will be achieved by reducing the need for antibiotics. Certain pig farmers will have to implement multiple changes to their production practices (e.g. improved and modified diets, later weaning, and increased space per pig) together with adapting the feed mixing /delivery systems to permit the targeted delivery of in feed medication to reduce the volumes used. Increased use of laboratory confirmed diagnosis, alternatives to antibiotics and a rigid herd health plan are all essentials if progress is to be made. The impossible is not been asked for as dramatic reductions in antibiotic use have been achieved in some EU countries and in many countries there are farmers which have demonstrated that diseases can be controlled without the need for antibiotic overuse. If the entire industry adopts the current best practice the pig industry will be seen to play its part. Robust biosecurity has be become the norm and those pig producers who won’t engage will have to be culled from the sector because they will only draw adverse publicity and draconian legislation on the entire industry.

Abstracts of Oral Presentations

Oral Abstracts - Wednesday 08 June 2016

NEW VIRUSES

O-VVD1-001

Development Of Congenital Tremors Following Novel Pestivirus Inoculation

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Introduction: Congenital tremors (CT; *myoclonia congenita*) is a sporadic but globally distributed disease of neonatal pigs characterized by action-related repetitive myoclonus. The first reports of the disease date back nearly one hundred years, yet most contemporary cases of CT are clinically ascribed to an unidentified virus. The aims of this project were twofold: 1) to identify potential pathogen(s) using next generation sequencing in cases of CT, and 2) to develop an innovative inoculation model to reproduce CT.

Materials and Methods: Next generation sequencing was performed on tissues from two field cases of CT. A novel agent was detected. For the second aim, seven individually identified crossbred sows were randomly assigned to one of three separately-housed groups: 1) sham-inoculated at 45 days gestation (n=1) and 62 days gestation (n=1), 2) pestivirus-inoculated at 45 days gestations (n=2), and 3) pestivirus-inoculated at 62 days gestation (n=3). Sows were anesthetized and the uterus exteriorized. A handheld linear array ultrasound was used to visualize each fetus and to inject each fetal vesicle with 0.25mL of either PBS (sham) or pestivirus-laden serum. Sows were also inoculated simultaneously by intravenous and intranasal routes per protocol. Sows farrowed normally. Video of each uniquely-identified piglet was taken at 0, 24 and 48 hours post-farrowing. Tremor severity was evaluated blindly by four investigators whereby each piglet received an averaged tremor severity score. Piglets were humanely euthanized and necropsied at 48 hours post-farrowing.

Results: A novel virus most closely related to a Chinese bat pestivirus at the time and now more closely related to a recently published novel porcine pestivirus was discovered from field cases of CT. Pestivirus RNA was detected by PCR in multiple tissues from piglets with CT but not in unaffected piglets from three separate farm investigations. Neonatal piglets from groups inoculated with pestivirus at both 45 and 62 days gestation were affected with CT, with prevalence within affected litters ranging from 57 to 100%. Tremor severity varied from mild to severe. Pestivirus RNA was consistently detected by PCR in numerous tissues at necropsy including serum, whole blood, cerebrum, cerebellum, brainstem, spinal cord, mesenteric lymph node, and tracheobronchial lymph node. Congenital tremors were not observed in control piglets and pestivirus RNA was not detected by PCR.

Conclusion: This is the first report to 1) identify a novel porcine pestivirus associated with CT and 2) successfully reproduce CT following inoculation of fetuses in utero.

Disclosure of Interest: None Declared

Keywords: Congenital tremors , myoclonia congenita, novel pestivirus

NEW VIRUSES

O-VVD1-002

Congenital tremor Type A-II in newborn piglets is caused by transplacental transmission of a novel pestivirus

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Introduction: Congenital tremor is a well-known phenomenon in newborn piglets. It is characterized by tremors of the head and limbs that vary in severity, but are reducing or even absent during sleep. They last for several weeks to months but decrease in severity as the pigs grow older. Historically, congenital tremor has been classified as type A, with defined pathological characteristics and partially known etiology, or type B, with unknown etiology. Type A is further divided into five subgroups, based on the different causes of congenital tremor and pathological characteristics. Congenital tremor type A-II remained the most puzzling subgroup, but has been regarded as a transmissible disease since the 1970's, probably caused by a virus. So far the virus involved remained elusive.

Materials and Methods: A next generation sequencing platform, VIDISCA (Virus discovery cDNA-AFLP) together with Roche-454 nucleotide sequencing was used to identify a possible novel virus. Serum samples from diseased piglets were compared with samples from healthy piglets. A quantitative RT-PCR was set up for rapid identification of all variants of the identified virus so far, based on amplification of a conserved region of the virus in the 5'UTR. Gilts were experimentally inoculated with serum from infected pigs.

Results: We identified a novel pestivirus in serum of piglets with congenital tremor in 2012. In the 2012-2015 period, several variants of this pestivirus were discovered on eight farms that are periodically affected by the disease. Piglets on two farms with no history of congenital tremor type A-II were negative for the virus. The virus was found in 83 out of 83 piglets with clinical signs of congenital tremor A-II. In order to demonstrate the causal relationship between pathogen and disease, three gilts were experimentally infected with the virus at day 32 of gestation. In two of the three litters born from these gilts, several piglets presented with mild to moderate clinical signs of congenital tremor type A-II, while piglets of one litter were symptom-free. Exactly the piglets born in this last litter were all free of the novel pestivirus, whereas the piglets with clinical congenital tremor type A-II from the affected litters were all positive for the virus.

Conclusion: We conclude that transplacental transmission of the new pestivirus, which we propose to be named congenital tremor associated porcine pestivirus (CT-APPV), is causing congenital tremor type A-II in piglets. We are now setting up serological and PCR analyses to further investigate the prevalence of CT-APPV in the Dutch pig population, and the effect on farm performance parameters.

Disclosure of Interest: A. De Groof Conflict with: Research supported by MSD AH, M. Deijs: None Declared, L. Guelen: None Declared, L. van Grinsven: None Declared, L. van Os-Galdos: None Declared, W. Vogels: None Declared, C. Derks: None Declared, T. Cruijsen: None Declared, V. Geurts: None Declared, M. Vrijenhoek: None Declared, J. Suijskens: None Declared, P. van Doorn: None Declared, L. van Leengoed: None Declared, C. Schrier: None Declared, L. van der Hoek Conflict with: Research supported by MSD AH

Keywords: congenital tremor, pestivirus

NEW VIRUSES

O-VVD1-003

Identification of novel Senecavirus A from pigs with vesicular disease in the US

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Introduction: Senecavirus A (SV-A), also known as Seneca Valley virus (SVV), belongs to the *Picornaviridae* family. The prototype SVV-001 was initially identified as a contaminant from the PER.C6 cell line. Since then, the virus has been detected infrequently in pigs with idiopathic vesicular disease (IVD) which grossly resembles FMD, swine vesicular disease, vesicular exanthema of swine and vesicular stomatitis in the US. In 2015, IVD cases increased dramatically. At the same time Brazilian researchers reported transiently neonatal mortality syndrome with IVD associated with detection of SV-A in a large number of swine breeding farms. We are reporting identification and isolation of novel SV-A from vesicular disease outbreaks in both exhibitions and commercial farms in the Midwest of the United State in 2015.
Materials and Methods: Starting from July, 2015, IVD outbreaks in show and commercial pigs in Iowa and South Dakota and then other states were reported without apparent epidemiological link. Affected animals suffered from acute lameness due to vesicular lesion on foot pad and coronary band, accompanied by anorexia and pyrexia. Gross lesion were characterized as coronary band hyperemia, cutaneous vesicle formation resulting in rupture and ulceration. Vesicles were developed on snout and/or in the oral cavity. Because of clinical signs, samples of all cases were submitted to the USDA for various vesicular FAD such as FMD, VSV, SVD. The samples were then tested by conventional and molecular assays for common swine pathogens at Iowa State University Veterinary Diagnostic Laboratory.
Results: All case materials were negative for the FAD agents but positive for SV-A. VP1 and full-length genome sequencing of the SV-A isolates from the index cases revealed that all the isolates shared 99-100% homology to each other but were significantly divergent from early SV-A sequences (1988-2001) deposited in the GenBank. The US SV-A isolates shared 97-98% homology in genome sequences with Brazilian SV-A isolates.
Conclusion: Laboratory testing data suggest that SV-A was likely responsible for IVD outbreaks. Genetically all of SV-A detected/isolated were closely related to each other but significantly different from previously reported SV-A. Phylogenetic analysis of the genome and VP1 sequences showed the SV-A has been evolving during the almost past three decades and achieved particular traits of enhanced pathogenicity. SV-A needs to be included when vesicular diseases are investigated.

Disclosure of Interest: None Declared

Keywords: Senecavirus A

NEW VIRUSES

O-VVD1-005

Novel RNA-based in situ hybridization for detection of Senecavirus A in pigs

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Introduction: Senecavirus A (SVA) has been recently associated with vesicular disease and neonatal mortality in swine. The assumption of SVA being the causative agent of disease had been based on virus detection by PCR associated with clinical signs of vesicular lesions. Primary antibodies against SVA are not commercially available. In situ hybridization (ISH) is a nucleic acid-based method that allows the detection of a particular RNA or DNA sequence within the tissue sections. A novel ISH RNA-based chromogenic technique (RNAScope) has been recently developed and uses a hybridization-based signal amplification system. The objective of this study was to evaluate the performance of this ISH-RNA to detect SVA within the lesions of affected sows and piglets.
Materials and Methods: A total of 30 Formalin-fixed paraffin embedded tissues were retrospectively selected based on positive PCR for SVA, from Veterinary Diagnostic Laboratory in University of Minnesota (MNVDL) cases. Samples were collected from sows with vesicular disease and also from piglets with acute neonatal mortality. Probe targeting specific genomic regions of the SVA (VP1 gene) were developed based on validated PCR probes, which are currently used at MNVDL. Hybridization signals were detected as red colorimetric staining followed by counterstaining with hematoxylin. Ten PCR negative samples from SVA from non-affected animals were used as negative controls.
Results:
Vesicular Disease. SVA was demonstrated in skin tissues of sows showing vesicular disease. SVA was predominantly located in microvesicular lesions in the *stratum spinosum* of the epidermis. Skin sections from non-affected animals were negative.
Neonatal mortality. Sixteen PCR positive tissues including skin, spleen, liver, heart, small intestine and lymph node were also positive by ISH-RNA. All tissues from non-affected piglets were negative. Despite the positivity on PCR and ISH-RNA, the only lesion observed in piglets showing sudden death was necrotizing glossitis. The presence of SVA was observed within these lesions.
Conclusion: The present study represents the first report demonstrating SVA within vesicular lesions in affected sows and within different tissues in affected piglets. We also first demonstrated SVA associated with necrotizing glossitis in acutely death piglets previously positive for SVA by PCR.
Finally, the referred ISH-RNA showed promising results regarding its applicability for a rapid diagnostic response, especially when there are no antibodies available for IHC. This information will help to optimize the sample collection for SVA investigation and increase the ability to diagnose the disease.

Disclosure of Interest: None Declared

Keywords: Diagnostic, In situ hybridization, Senecavirus A, vesicular disease, neonatal mortality

Oral Abstracts - Wednesday 08 June 2016

NEW VIRUSES

O-VVD1-004

Vesicular Disease in 9-week-old Pigs Experimentally Infected with Senecavirus A

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Introduction: Senecavirus A (SVA), a picornavirus, has been infrequently associated with cases of idiopathic vesicular disease (IVD) in pigs in the US and Canada since 1988. In 2014 and 2015 there was surge of IVD cases in Brazil and US, respectively. SVA was identified in serum, vesicular fluid, and ruptured vesicles collected from affected pigs. Presumably, SVA was the cause of these recent epidemics; however, Koch's postulates for this disease have not yet been fulfilled.
Materials and Methods: Nine-week-old pigs (n=29) received an intranasal inoculation of SVA15-41901SD isolate ($5x10^7$ pfu/pig) at 0 days-post-inoculation (dpi). Twelve pigs (Dex-SVA) were treated with an immunosuppressive dose of dexamethasone for 5 days prior to challenge. Serum and swab samples were collected at regular intervals and tested by PCR, SVA-antibody (serum) and virus isolation. A randomly selected SVA pig was euthanized and necropsied at 2, 4, 6, 8, and 10 dpi.
Results: At 5 dpi, 24 of the remaining 27 infected pigs had intact or ruptured vesicular lesions on the coronary bands of toes and dewclaws and/or the interdigital cleft of one or more feet without causing severe lameness. A subset of animals developed erosions on the lower lip and snouts (after 10 dpi). Dex-SVA pigs developed slightly larger vesicular lesions than the untreated pigs and were observed around one day earlier. At 3 dpi SVA was detected in serum from each pig by PCR and in all swab samples collected from vesicular lesions at 5 dpi regardless of Dex treatment. All pigs seroconverted to SVA by 15 dpi as determined by indirect fluorescent antibody test. SVA was also isolated from vesicular fluid.
Conclusion: Vesicular disease was experimentally induced in nursery-age pigs inoculated with SVA demonstrating for the first time a causal relationship between SVA infection and disease. This is important because SVA disease is clinically indistinguishable from foot-and-mouth disease, which is induced by a highly transmissible picornavirus that can cause devastating economic losses to the agricultural industry and human food supply. Therefore a better understanding of SVA pathogenesis and host response to infection should aid in the development of prevention and control measures and differentiation of this virus from other vesicular diseases.

Disclosure of Interest: None Declared

Keywords: nursery pigs, Senecavirus, Vesicular lesions

PRRS

O-VVD2-009

Characterization of a PRRSV isolate bearing strong homology to a Chinese vaccine strain

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) is still a widely disseminated pathogen of swine in the world. In 2006, the highly pathogenic PRRSV (HP-PRRSV) that caused high morbidity and mortality of swine emerged in China, and has become the dominant strain of prevalent PRRSVs here. Since 2012, HP-PRRSV-derived commercial live vaccines have been widely used in China. Thus, multiple PRRSV strains co-exist in the swine herds and have caused much concern about virulence reversion or potential virus recombination. Jiang et al (2015) reported three revertants of the HP-PRRSV vaccine strain JXA1-P80 in south-east China. Here, we report another virulence-reversed vaccine strain HENZK-1 isolated from a pig farm in central China that appears to be a revertant of the vaccine strain JXA1-R, confirming the considerable risk of the virulence reversion of HP-PRRSV-derived live vaccine.
Materials and Methods: Tissue samples of acutely ill sows were collected from a swine herd affected by the reproductive problems in sows and markedly increased mortality in piglets in Zhoukou, Henan province, China, and passaged in MARC-145 cells. RT-PCR was performed using 14 pairs of primers based on the HP-PRRSV strain JXA1. The amplified PCR products were cloned into the pMD18-T vector for a full-length sequencing. The nucleotide and deduced amino acid sequences were analyzed by DNAstar, and phylogenetic trees were constructed by MEGA5 along with the reference strains.
Results: The sample was identified as PRRSV-positive by using RT-PCR as well as IFA. The isolate was named as HENZK-1. The sequencing results showed that the isolate genome had a discontinuous deletion of 90 nucleotides in the Nsp2 coding region normally considered to be a unique gene marker of the HP-PRRSVs. More importantly, we found that the isolate HENZK-1 shared the highest nucleotide identity (>99%) with the JXA1 derivatives.
Conclusion: In this study, PRRSV was isolated from a 70-sow herd 21 days post inoculation with HP-PRRSV vaccine strain JXA1-R. In this herd, ten percent of 60-day old pigs were affected by high fever and cough as well as death. Genomic sequence analysis showed that the isolate shared very high homology (>99%) with JXA1-P80, a vaccine strain developed by passaging strain JXA1 in MARC-145. Thus, we reasonably consider HENZK-1 as a revertant of the live HP-PRRSV vaccine strain JXA1-R as an atavism of virulence and this should cause much concern about the use of attenuated HP-PRRSV live vaccine in the field.The study on the pathogenicity of the isolate is under conduction.

Disclosure of Interest: None Declared

Keywords: vaccine strain , virulence-reverse, HP-PRRSV

PRRS

O-VVD2-006

Evaluation of time to stability and associated risk factors in sow herds infected with PRRS 1-7-4

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Introduction: Porcine Reproductive and Respiratory Syndrome Virus (PRRSv) 1-7-4 has emerged within the United States swine industry since 2014. Herds infected with PRRS 1-7-4 result in reduced reproductive and growth performance. A previous cohort study determined that the median time to stability (TTS) for 61 herds infected with various PRRS strains was 26.6 weeks, ranging from 12-42 weeks. The objective of this study was to evaluate TTS and associated risk factors in PRRS 1-7-4 infected sow herds enrolled in the Swine Health Monitoring Project (SHMP).
Materials and Methods: All SHMP participants were invited to enroll breeding herds into the study that met a set of inclusion criteria. The criteria consisted of farrow-to-wean herds, diagnostic evidence of PRRS 1-7-4 infection, no diagnostic evidence revealing the herd being infected with a different PRRSv strain from the time of first detection to TTS, intent to produce PRRSv negative piglets (reach stability), and ≥ 30 weaning-age pigs from the herd were tested for PRRSv monthly starting no later than 13 weeks post PRRSv detection. TTS was defined as the date of the last negative test of at least 4 consecutive tests for a minimum of 90 days post PRRSv detection. The study consisted of 126 enrolled herds. Preliminary information regarding TTS and associated risk factors from 126 PRRS 1-7-4 positive sow herds were evaluated. A preliminary analysis of TTS and associated risk factors was achieved through survival and univariate analysis using Statistix 9.
Results: Of the 126 herds with complete data to date, 62 (49%) reached stability. The TTS ranged from 19 to 83 weeks. The median TTS was 36 weeks (mean 37.4), or 24 weeks when assessed from date of first negative test followed by three consecutive negative tests. When compared to the previous study of 61 herds, the cohort of preliminary 1-7-4 herds attained stability 2.6 weeks earlier. Modified live virus vaccination of breeding sources post PRRS detection, high weaning frequency, and specific system were significantly associated with longer TTS. Herds with onsite gilt development units (GDU) reached stability 5.5 weeks earlier (p=0.005).
Conclusion: Based on the conditions of this investigation, 49% of the enrolled herds reached stability at a median of 36 weeks. Variability of TTS among herds was observed based on sow herd vaccination, GDU location, system, and weaning frequency. Compared to the previous study, herds infected with PRRS 1-7-4 reached TTS 2.6 weeks sooner.

Disclosure of Interest: None Declared

Keywords: PRRSv 1-7-4, Time to stability

PRRS

O-VVD2-007

Factors associated with porcine reproductive and respiratory syndrome virus (PRRSV) infection dynamics in infected herds

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Introduction: PRRSV is considered to be one of the most important diseases affecting pigs worldwide. PRRS control only based on vaccination often provides results limited to clinical improvements. A thorough knowledge of PRRS epidemiology is required to implement efficient control strategies. In the field, different clinical outcomes can be observed according to infection dynamics. Factors associated with the age at PRRSV infection time have not been investigated to date. Thus, the aim of this study was to explore the factors related to PRRSV infection dynamics in infected herds.
Materials and Methods: The study was carried out in 65 farrow-to-finish French herds. Tracheo-bronchial mucus and blood were taken from a random sample of 4, 10, 16 and at least 22 week-old pigs (45 pigs/herd). Serum were tested by ELISA for PRRSV antibodies. Infection by *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae*, swine influenza viruses H1N1 and H1N2 and porcine circovirus of type 2 (PCV2) were detected by specific serological or PCR tests. Data related to husbandry, biosecurity, management and housing conditions were collected by a questionnaire. Climatic conditions in the nursery and fattening rooms, where the oldest sampled pigs were housed, were measured over 20 hours. The outcome in the statistical process was the age-time to PRRSV seroconversion. The within batch frequencies of seropositive pigs were used to estimate the time-interval during which seroconversion was deemed to occur in each herd. Factors related to the age-time to PRRSV seroconversion were identified by a Cox proportional hazards model.
Results: A common housing for the gilts and the sows during lactation (Hazard Ratio [HR]=3.0; $IC_{95\%}$[2.0-4.3]), large sized nursery pens (HR=2.9; $IC_{95\%}$[2.0-4.1]), a small number of pens per fattening room (HR=2.5; $IC_{95\%}$[1.7-3.6]) and the lack of all-in all-out management in the fattening section (HR=2.5; $IC_{95\%}$[1.8-3.4]) significantly reduced the age-time to seroconversion. A small range of temperatures controlling ventilation in the nursery room was associated with an early PRRSV seroconversion (HR=3.9; $IC_{95\%}$[2.8-5.4]). Infection by *M. hyopneumoniae* (HR=3.2; $IC_{95\%}$[2.3-4.5]) and a high PCV2 infection pressure (HR=4.6; $IC_{95\%}$[3.1-6.9]) were also related to early PRRSV seroconversion.
Conclusion: Non-infectious and infectious factors were found related to PRRSV early infection and thus to herd instability. It suggests associating management practices minimizing direct and indirect contacts between animals within batches and from different batches whilst providing the pigs with favourable climatic conditions for a better control of PRRSV infection in swine herds.
Disclosure of Interest: None Declared
Keywords: Epidemiology, Infection dynamics, PRRS

Oral Abstracts - Wednesday 08 June 2016

PRRS

O-VVD2-008

Applying Bioportal in a PRRS Regional Disease-Control Program: case study

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Introduction: Introduction of gilts into a swine farm always conveys the risk of disease introduction into the recipient herd and, consequently, from a regional perspective, disease introduction into clusters of neighboring farms. In several areas in North, Central and South America, the swine industry is organized in projects of PRRS (Porcine Reproductive and Respiratory Syndrome) control by regions. These projects allow for coordinated actions to control PRRS and for sharing of information regarding swine diseases circulating in the area. Information related to diseases present in the different regions can be analyzed simultaneously in time, space and genomics applying a program called Bioportal which was originally developed at UC Davis to understand the dynamics of Foot and Mouth Disease worldwide.
Materials and Methods: In this case, 500 replacement gilts imported into the country tested PCR and ELISA negative to PRRS virus (PRRSv) upon shipment, arrival and seven days into quarantine. Three weeks after arrival, the gilts tested PCR PRRSv positive. When sequenced, a 1-26-2 RFLP cut pattern was detected. In order to assess if this PRRSv isolate was a new introduction to the region or a resident virus, those nucleotide sequences were compared against the available regional database. Bioportal was applied to generate a dendrogram linking sequences on it to a map of their corresponding sites of origin. The swine industry usually applies a threshold of ≥2% difference on the ORF5 region of the PRRSv to determine similarity among sequences.
Results: The resultant dendrogram showed three different branches: one at >6% difference –heterology-, another at between 2 and 6%, and one more at < 2% heterology from the case's sequence. Sixty eight (n=68) isolates were included in the branch showing < 2% heterology to the case's isolate. The map in that report showed that those 68 isolates were located in the same sub-region within the project's area.
Conclusion: The agreements reached to share diagnostic data among participants from projects of regional control of PRRS have shown great value when investigating questions related to the dynamics of pathogens' presentation and distribution in those regions. In this case, the availabilty of such data and the application of a powerful tool as Bioportal allowed the management of large amounts of data (e.g., geographic location, nucleotide sequences, different points in time, etc.) to generate analytical and visual/descriptive reports in a fast, timely approach. Once determined that the PRRSv isolate from the quaratined gilts was a regional resident virus, an investigation was conducted to understand routes of lateral introduction.

Disclosure of Interest: None Declared

Keywords: ARC Project, Bioportal, PRRS

PRRS

O-VVD3-010

Maternally-derived antibodies impair piglet humoral and cellular immune responses to vaccination against porcine reproductive and respiratory syndrome

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Introduction: Porcine Reproductive and Respiratory Syndrome (PRRS) is among the most costly diseases for swine industry worldwide. Vaccination is one of the tools used to limit the economic impact of the disease. The most commonly used vaccines are modified live vaccines (MLV) which have proven their efficacy to limit clinical consequences of PRRS in breeding herds as well as in growing pigs. We recently showed that MLV can also efficiently reduce virus transmission in growing pigs under experimental conditions; however the same efficacy seems difficult to achieve in the field. We hypothesized that this discrepancy could be due to the presence of maternally derived antibodies (MDA) which might decrease vaccine efficacy in piglets under field conditions.
Materials and Methods: The influence of MDA on piglets' immune response to PRRS vaccine was studied in a herd without PRRS virus (PRRSV) circulation but with PRRS vaccination in the breeding herd. Thirty piglets with a low (A-) or high level (A+) of maternally derived neutralizing antibodies (MDNA) were vaccinated (V+) with a genotype 1 MLV at 3 weeks of age. Blood samples were collected before vaccination and then at 2, 4, and 8 weeks post-vaccination (WPV). The samples were analyzed to detect the vaccine viremia (RT-PCR) and to quantify the post-vaccination humoral (ELISA and virus neutralization test) and cellular (ELISPOT IFNɣ) immune responses.
Results: PRRS vaccine genome was detected in 60%, 64% and 36% of A-V+ piglets at 2, 4 and 8 WPV, respectively. On the contrary, no virus was detected in A+V+ piglets during the first 4 WPV but 32% were PCR positive at 8 WPV. 85% of A-V+ piglets and 0% of A+V+ piglets seroconverted (ELISA) between 2 and 4 WPV. Neutralizing antibodies appeared 4 WPV in A-V+ piglets but were not detected at 8 WPV in A+V+ piglets. The number of PRRS-specific IFNɣ secreting cells was significantly higher in A-V+ piglets compared to A+V+ piglets with respectively a mean of 216 and 10 cells/million PBMC at 2 WPV and 214 and 4 cells/million PBMC at 4 WPV.
Conclusion: These results show for the first time that MDNA can impair both humoral and cellular immune responses in piglets vaccinated against PRRS, probably by inhibiting replication of the PRRS vaccine strain. Further studies are now required to assess the impact of MDNA on vaccine efficacy following a PRRSV challenge.

Disclosure of Interest: None Declared

Keywords: Maternal Antibody, PRRS MLV vaccine

PED

O-VVD3-013

Pathogenicity and Cross-protective Immunity of the United States PEDVs

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Introduction: In North America, porcine epidemic diarrhea virus (PEDV) was detected for the first time in the US in April 2013. Herein we summarize some features of pathogenicity and cross-protective immunity of US PEDVs based on work conducted in our laboratory.
Materials and Methods: STUDY 1 (S1). To determine the oral minimum infectious dose (MID) of PEDV, a US PEDV prototype isolate with known infectious titer was serially diluted and orogastrically inoculated into 7 groups of 5-day-old neonatal pigs (n=4/group) and 7 groups of 21-day-old weaned pigs (n=6/group).
S2. To compare the pathogenicity of US PEDVs, 50 5-day-old pigs were randomly divided into 5 groups (10 pigs/group) and orogastrically inoculated (10^5 $TCID_{50}$/pig) with one of 3 US PEDV prototype isolates, or a US PEDV S-INDEL-variant isolate, or virus-free culture medium.
S3. To explore the serological cross-reactivity and cross-neutralization of the US PEDV prototype and variant strains, experimentally-generated antisera against the two strains were evaluated using 5 assays: 1) PEDV IFA antibody assay; 2) virus neutralization test; 3) prototype whole virus-based ELISA; 4) prototype S1-based ELISA; and 5) variant S1-based ELISA.
S4. To examine the cross-protection efficacy between two US PEDV strains, 85 PEDV-naïve 3-wk-old pigs were divided into 7 groups with 15 or 10 pigs per group and inoculated with culture media, PEDV prototype isolate, or PEDV variant isolate at Day 0 (D0) followed by challenge at D28.
Results: S1. In neonatal pigs, 10ml of inoculum having titers 560-0.056 $TCID_{50}$/ml (Ct 24.2-37.6) caused 100% infection and titer 0.0056 $TCID_{50}$/ml (Ct>45) caused infection in 25% of inoculated pigs. In weaned pigs, 10ml of inoculum with titers 560-5.6 $TCID_{50}$/ml (Ct 24.2-31.4) caused 100% infection while titers 0.56-0.0056 $TCID_{50}$/ml did not establish infection. The data indicated that PEDV infectious dose is age-dependent with a significantly lower MID for neonatal than weaned pigs.
S2. All 3 US PEDV prototype isolates caused severe enteric disease in 5-day-old pigs. In contrast, the US PEDV variant isolate caused much milder enteric diseases as compared with the prototype isolates.
S3. Antibodies against the US PEDV prototype and variant strains cross-reacted and cross-neutralized the two strains *in vitro*.
S4. US PEDV prototype strain appeared to be more virulent than variant strain in 3-wk-old pigs but opposite in 7-wk-old pigs in this study. US prototype PEDV provided protection against challenge with prototype or variant strains; US variant PEDV provided protection against variant strain challenge and at least partial protection against prototype strain challenge in weaned pigs.
Conclusion: Included in Results section.

Disclosure of Interest: None Declared
Keywords: None

PED

O-VVD3-012

Comparison of porcine epidemic diarrhea virus (PEDV) and porcine deltacoronavirus (PDCoV) for pathogenicity in nursery aged pigs

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Introduction: The coronavirus, transmissible gastroenteritis virus (TGEV) has been a major enteric pathogen of US swine since 1946. The emergence of PEDV and PDCoV in the US between 2013 and 2014 created awareness to their economic impact. While pathogenicity of these individual enteric coronaviruses in pigs, particularly at pre-weaning ages, has already been documented, there is no report comparing pathogenicity of each swine virus against each other. Therefore, we evaluated the pathogenicity of these two swine coronaviruses simultaneously.
Materials and Methods: Eight week old pigs, naïve for PEDV, PDCoV, and TGEV, were allotted to one of the following treatment groups (n = 25 pigs/group): 1) Control; 2) PEDV inoculated; and 3) PDCoV inoculated. At 0 day post inoculation (dpi), all pigs received a gastric gavage of their designated viruses (10^3 $TCID_{50}$/ml) or a sham inoculum for the control group. Serum, feces, and oral fluids were sampled from each pig daily during the first 5 dpi. Subsequently, weekly sampling occurred. In addition, body weight and feed intake were recorded weekly to monitor growth performance of the pigs per treatment group. Clinical signs and the severity of clinical disease were also monitored.
Results: Pigs in both of the virus groups displayed signs of watery diarrhea or lose stool starting 1-2 dpi. The PEDV infected pigs also displayed signs of lethargy and anorexia, but the PDCoV infected pigs behaved similar to the control pigs. All animals recovered from diarrhea after 5 dpi. All infection treatment groups were detected positive by quantitative RT-PCR. After 2 dpi, differences were observed in the virulence and viral shedding between the treatment groups. PEDV was detectible in pigs for up to 7 dpi, while PDCoV was only detectible in infected pigs at 3 dpi. PEDV infected pigs had significant reduction in growth performance based on ADG and ADFI compared to control pigs. There was no significant impact on performance for the PDCoV infected group. In the gastrointestinal tract, PEDV RNA was most persistently present in both the small and large intestinal tissues with the highest viral load when compared to the PDCoV group.
Conclusion: Overall, the pigs infected with PEDV had more severe clinical manifestations including growth performance with the greatest viral load, while the PDCoV infections were less severe under the conditions of this investigation.

Disclosure of Interest: None Declared

Keywords: comparison, Coronavirus, Porcine epidemic diarrhea virus

Oral Abstracts - Wednesday 08 June 2016

PED

O-VVD3-011

Applied strategies to prevent spread of swine enteric coronaviruses in a North American multiplication system

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Introduction: Porcine Epidemic Diarrhea (PED) and Porcine Delta Coronavirus (PDCoV) entered the US swine population in 2013. Though some production systems had developed successful biosecurity programs (including remote locations) to prevent spread of PRRS and *Mycoplasma hyopneumoniae*, these strategies were not sufficient to thwart introduction of these Swine Enteric Coronaviruses (SECs). Specifically, between November 2013 and April 2014, 47.4% of PIC North American breeding herds became infected with SECs, while 56% of US sow herds were infected. Though PIC-NA successfully eliminated SECs in all affected breeding herds, new and more aggressive strategies were needed to prevent further re-infection and spread of SECs in the PIC system.
Materials and Methods: By the end of 2014, an SEC prevention initiative was fully implemented across the PIC multiplication system focused on mitigating risk in five main areas: (1) feed ingredients, reception, manufacturing and delivery, (2) transportation decontamination and inspections, (3) training and engagement of personnel, (4) manure management and (5) mortality disposal. PIC risk assessments for farms, feed mills and transport decontamination facilities were updated and corrective action plans initiated where needed. To increase biosecurity and risk awareness, educational materials were developed and disseminated through Internet. Each breeding herd or flow designated a biosecurity officer and monthly interactive webinars were utilized. An intensive surveillance program was initiated in early 2014 to minimize the risk of moving infected breeding stock. Surveillance included (a) daily evaluation of clinical signs by trained personnel, (b) routine testing based on oral fluids PCR and (c) contingency diagnostic investigations and cessation of breeding stock movements when clinical signs or unexpected diagnostic results were observed.
Results: New and intensified strategies were applied across the PIC system starting mid-summer 2014 with particular emphasis during high-risk seasons (September through April). SEC eliminations were 100% successful and complete in December 2014. No PIC sow herds were infected with SEC after April 2014 through time of submission.
Conclusion: Though biosecurity measures prior to 2013 were highly successful preventing the introduction of common pathogens to North American PIC herds, those measures were far less successful preventing the introduction of SECs. Prompt implementation focused on five biosecurity areas in addition to increased surveillance and utilization of site-specific biosecurity officers prevented the introduction of SECs to any sow herd in the system since April 2014.

Disclosure of Interest: J. Geiger Conflict with: PIC employee, J. P. Cano Conflict with: PIC employee, J. W. Lyons Conflict with: PIC employee, R. Thompson Conflict with: PIC employee, T. Riek Conflict with: PIC employee, T. Snider Conflict with: PIC employee

Keywords: PED, Prevention, SEC

O-REP1-001

Estimation of colostrum consumption of the neonatal piglets in three commercial swine herds in Thailand

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Introduction: Colostrum is the first milk secreted by the mammary gland of the sow from 0 up to 24 h postpartum. Colostrum is a source of digestible nutrients and various compounds, e.g., immunoglobulins, hormones and growth factors. It has been suggested that each piglet must consume at least 200–250 g of colostrum to ensure an optimal growth and passive immunity. The present study aims to determine colostrum consumption of the neonatal piglets in 3 commercial swine herds in Thailand.
Materials and Methods: The study was performed in 3 commercial swine herds in Thailand (A, B and C). In total, 2,399 piglets (986, 848 and 565 piglets in herds A, B and C) from 217 Landrace x Yorkshire crossbred sows were investigated. Farrowing supervision was performed in all litters for 24 h. Bodyweight of the piglets was measured at 0 and 24 h after farrowing by using electronic balance. Individual colostrum consumption of the piglets was estimated [colostrum consumption (g) = -217.4+(0.217*t)+(1,861,019*BW24h/t)+BW*(54.8-1,861,019/t)*((0.99853.7 *10^{-4}*tFS)+(6.1*10^{-7}*tFS^2)); where BW=birth weight (kg), BW24h=body weight at 24 h after birth (kg), t= time elapsed between the first and the second weighting (min), and tFS = the interval between birth and first sucking (min)]. Descriptive statistics and frequency analyses were used to analyze colostrum consumption of the piglets. Chi-square analysis and ANOVA was used to analyzed the difference of colostrum consumption of the piglets among herds.
Results: The average colostrum intake of the piglet was 279±141 g (range 0-940). Of all the piglets (n=2,399), 93 piglets (3.9%) received no colostrum and 651 piglets (27.1%) had colostrum consumption <200 grams. Piglets with colostrum consumption of 0-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500 and ≥500 grams were 7.0%, 4.4%, 6.2%, 9.5%, 14.5%, 14.6%, 14.0%, 11.9%, 7.1%, 5.2% and 5.6%, respectively. The piglets without colostrum consumption were 4.2%, 2.6% and 5.3% in herds A, B and C, resp. (P<0.05). The piglets with colostrum consumption ≤200 grams were 30.8, 27.7 and 19.8% in herds A, B and C, resp. (P<0.001). The average colostrum consumption were 267, 277 and 300 g in herds A, B and C, resp. (P<0.001). These data indicates that,
under field conditions, a certain number of piglets consume suboptimal amount of colostrum and may subsequently increase the risk of pre-weaning mortality.
Conclusion: Under field conditions, 3.9% of the neonatal piglets did not received any colostrum and 27.1% of them received suboptimal level of colostrum (i.e., ≤200 g). Thus, factors associated with the piglet colostrum consumption under field conditions should be carefully investigated.

Disclosure of Interest: None Declared

Keywords: Colostrum, Piglet, Pre-weaning mortality

O-REP1-002

Incidence and fertility of sows with lactational estrus in a mid-size commercial sow farm with Danish genetic

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Introduction: Sows in lactation are believed to be anestrous due to suckling-induced suppression of LH/FSH- secretion and thus, no follicle growth and ovulation occur. Recent research and field observations, however, indicate that, lactational estrus (LE) occurs, and management tools such as split-weaning has been used to purposely induce LE. The aim of this study was to determine the incidence of LE in a farrow-wean sow farm with Danish genetic, and to analyze production data of sows bred in their LE.
Materials and Methods: The farm had an inventory of 2,200 sows/gilts and a weekly batch farrowing system with on average 100 sows per batch, and was located in Saxony, Germany. Data was collected for the period January–May 2015. A total of 16 batches with 1,563 sows were evaluated. Average lactation length was 26 days. Heat checking in lactation started at the end of the third week and was continued through the fourth week daily with the help of “boar spray”. Sows found with LE were moved to the breeding area where they were bred on an AM/PM schedule with two AI’s/sow. Normally weaned sows (1,154) got boar exposure starting the day post weaning and were bred as reported for sows with LE. Pregnancy (PR) & farrowing rates (FR) as well as litter sizes of sows bred in LE and normally weaned were recorded.
Results: Number of sows with LE was 66 (4.2 %). There was no parity effect (range: 1-10; mean: 3.7). Batches varied in the percentage of sows with LE (0-12.1%). LE occurred on day 22 of lactation on average (range: 14-27). Mean PR and FR were 80.3% and 71.2% (range PR: 40-100%; range FR: 40-100%). Mean total and liveborn piglets were 17.7 and 14.9 (range: 4-26 and 3-21). For normally weaned sows (parity 1-11; mean 3.6), mean PR was 91.6% and FR 85.7%. Mean total and liveborn piglets were at 17.9 and 15.6.
Conclusion: Study indicates that LE i) occurs starting in the third week of lactation, ii) irregularly fluctuates among batches, iii) does not have a parity effect and iv) when used for breeding results in a reasonably fertility. While using LE for breeding will decrease the number of non-productive days the overall benefit of this breeding practice remains to be determined considering e.g. production data and labor costs.

Disclosure of Interest: None Declared

Keywords: Sow, lactational estrus, fertility

Oral Abstracts - Wednesday 08 June 2016

O-REP1-003

Positive effect of oxytocin on placenta expulsion

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Introduction: With increasing litter size, management of the sow around farrowing has become critical. It is well known that use of oxytocin in conjunction with dystocia such as inertia uteri will enhance uterine contractions and use of exogenous oxytocin is therefore indicated. However, only very little has been reported about the use of oxytocin during the third phase of parturition, that is the expulsion of the placenta. We therefore hypothesized that use of oxytocin at the end of the second phase of parturition would improve the expulsion of the placenta and therefore decrease the overall duration of parturition.
Materials and Methods: We designed a trial with 107 crossbred sows (Yorkshire x Landrace, of parity 4,2 ± 1,1 SD) submitted to farrow. We grouped sows according to the use of oxytocin; 42 of the sows received one injection of oxytocin (10 IU i.m. 5 cm caudal from the ear base) at the end of the phase two of parturition. The remaining 65 sows served as controls. The complete process of farrowing was video recorded for the different phases of parturition, focusing on the expulsion of the placenta. On d 3 of lactation, a transcutaneous ultrasound examination (10 MHz, Esaote SpA, Italy) was applied for determination of the presence or absence of placenta as well as the size of the uterus. We analyzed the effect of use of oxytocin on farrowing – related, fetal and uterine parameters with independent two sample t-test and chi-square test where appropriate (PASW Statistics v. 18.0.0).
Results: The sows farrowed an average of 16,2 ± 3,8 born piglets / litter with an average duration of farrowing until the end of the second phase of 402,3 ± 244,0 min and an overall duration of farrowing of 695,3 ± 279,8 min. The average duration of expulsion of the placenta was 295,8 ±231,0 min. Use of oxytocin shortened the overall duration of farrowing by 136 min (P=0,013) and the duration of the expulsion of placenta by 119 min (P<0,01). The placenta was expulsed in 3,5 ± 1,4 parts, which was not affected by the use of oxytocin. According to the US scan post partum, use of oxytocin decreased retained placenta and tended to decrease the risk of increased uterine size (P<0,10).
Conclusion: The present results indicate that limited used of oxytocin at the end of the second phase of parturition may shorten the expulsion phase of the placenta and thereby the overall duration of farrowing. However, the number of parts of placenta expulsed was unaffected, suggesting an improved clearance of the uterus by endogenous oxytocin. The US findings indicate a potentially improved further clearance and involution of the uterus post partum by the use of oxytocin.

Disclosure of Interest: None Declared

Keywords: None

O-REP1-004

Update of toxic compounds from plastic packaging used in artificial insemination that cause reproductive failure

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Introduction: In intensive pig production systems, 100% of fecundation is performed using artificial insemination. Semen from boars is collected, diluted and finally placed into a high barrier plastic packaging until final use. Sperm quality is always checked during processing, and routine parameters are measured before insemination. Sperm is accepted for breeding use only if these parameters achieve the required thresholds. Reproduction efficiency in swine is high, but some failures are acceptable and the origin can be related to many reasons. From 2010 some reproductive failures have been associated with the migration of toxic compounds from bags to the semen. Recent studies have detected toxic compounds in heat sealing tubes too.

Materials and Methods: Analysis and characterization of the plastic blisters and tubes suspicious of reproductive failure in farms, migration study, sperm functionality tests, in vitro/in vivo fecundation test, endocrine profiling panel and post- mortem uterus flushing of inseminated sows were carried out. All the statistic tests were performed using StatView 5.0 software.SAS Institute Inc.
Results: Toxics found in the plastic blisters were octyl phthalate, erucamide and BADGE (bisphenol A diglycidyl ether), 1,4-trioxacyclotridecane-8,13-dione (lactone) and a phthalate with cyclic structure.
Toxic characterized in the heat sealing tubes was phthalate with cyclic structure.
Analysis of blisters pointed to the BADGE as primarily responsible for reproductive failures. Moreover a synergistic effect with the cyclic lactone, was observed.
In heat sealing tubes, the responsible of the failure was the phthalate.
Spermatozoa preserved in both systems passed all of the routine quality control tests (>70% total motility, > 50% HOST, > 80% acrosome integrity), and no differences were observed between storage in the control and suspicious packagings. In vitro fecundity tests showed differences only in the toxic coming from the tubes .EPP(endocrine profiler panel analysis) did not show any alterations. In vivo tests confirmed the failure in both systems.

Conclusion: This paper demonstrates that reproductive failures can occur in different kind of plastics even when internal quality control tests were not able to detect any alteration of quality parameters. Unfortunately, it was not possible to prevent the reproductive failure before this study, because the relationship between unexpected unknown substances and the reproductive failure was completely unknown and was thus, undetectable. This paper is the first description of the interaction between different compounds and reproductive failure.

Disclosure of Interest: None Declared

Keywords: None

O-REP1-005

The effect of Sperm Photostimulation by improving reproductive parameters with the use of Artificial Insemination

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Introduction: The use of artificial insemination (AI) with fresh semen is one of the key tools that have established the basis of exponential growth in world pork production last years.

The success of the AI is basically due to that it is an economical and easy technique to use in pig farms of any size. Furthermore, the reproductive performance achieved with AI is nearly optimal.

However, there are some unsolved problems that make the AI is not optimal for all commercial farms. One of these problems is the presence of a strong seasonality that affects fertility and prolificacy results.

The aim of this study is to find a tool that minimizes the effects of seasonality on AI in commercial farms and increases the fertility results in commercial farms.

Materials and Methods: Commercial refrigerated semen doses at 17ºC have been used.

Samples were subjected to an specific "in vitro" photostimulation procedure by using red LED sources in a regime that lasted 30 minutes. This procedure was adapted to a previously designed photostimulation chamber (maXipig®; Iul; Barcelona) that allowed to apply the procedure in "in vivo".

Following this, a total amount of 5620 sows were inseminated in 10 commercial farms between October 2014 and May 2015. For each batch of insemination in each farm, the multiparous sows have been divided in two groups:

- The Control batch was formed by sows with a conventional AI with refrigerated semen at 17ºC. - The LED batch corresponds to those sows that were inseminated with semen doses that were previously photostimulated by placing them inside the maXipig® chamber for 30 minutes.

Afterwards, both "in vivo" fertility and prolificacy results were collected.

Results: The "in vitro" tests showed a significant improvement of the overall sperm thermal resistance. Likewise, "in vivo" results showed a noticeable improvement of "in vivo" fertility data in all farms and at of each batch of insemination. The average value of improved fertility is 2.11%, but depending on farms, it oscillates between 1.15% and 11.52%.

Interestingly, the greatest effects were observed in those farms in which "in vivo" fertility data were the lowest at the start of the procedure.

There are not significant differences in prolificacy results (Born Alive and Total Born).

Conclusion: Results clearly indicate a net benefit of using the maXipig® photostimulation system on commercial farms to improve fertility results. This benefit is evident not only in seasonal periods in which fertility is affected, but also throughout year. Moreover, the improvement is reflected in both farms with good fertility results (fertility ≥ 90%) and in those farms with low fertility (≤85%), in which the increase in fertility is actually greater.

Disclosure of Interest: None Declared

Keywords: fertility improvement, LED system, photostimulation

O-REP2-006

Semen motility: a valuable tool for boar semen evaluation assessment at gene transfer centers

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Introduction: The relationship between boar semen quality characteristics and fertility can have a significant impact on the genetic merit of the offspring, gene dissemination and artificial insemination (AI) efficiency. The present study aimed to evaluate the relationship between boar semen quality and fertility (return rate: RR, farrowing rate: FR and number of total piglets born: TB) after on-farm homospermic AI.

Materials and Methods: Records from 1,455 ejaculates of 20 boars (PIC® terminal genotype) and 1,212 homospermic inseminations were used for analysis. The entire ejaculate from each boar was collected using the glove-hand technique once/week. Semen doses were prepared to contain 2.0 billion of viable spermatozoa in a total volume of 80 mL Androstar® (Minitub). Semen quality was assessed using CASA (SpermVision®, Minitub), through the analysis of 12 different motility parameters (Holt et al., 1997, J Androl, 18, 312). Boars were divided in two groups based on farrowing rate and litter size: Low fertility (LF, n=10: 70.4% FR, 17.3% RR and 8.9 TB) and High fertility (HF, n=10: 93.3% FR, 1.2% RR and 12.4 TB). Data were analyzed as a randomized complete design using the general linear model (GLM) procedure of SAS (SAS Institute Inc., Cary, NC). Least square means were compared using the Student T test with $P<0.05$ of significance.

Results: High fertility boars spermatozoa showed higher motility and progressive trajectory compared to their LF counterparts (highest LIN – 0,426 *vs* 0,395 and STR – 0,778 *vs* 0,729; $P<0.01$). Moreover, LF boars spermatozoa showed higher vigorous movement (highest VCL – 112 *vs* 110 µm/s, ALH – 3,4 *vs* 3,2µm and BCF – 30,3 vs 29,0 $P<0.01$) but following irregular trajectories (VCL > VAP).

Conclusion: As lower motility spermatozoa may limits the potential to fertilize oocytes, the present results suggest that semen motility can be a valuable tool to assess fertility information of boar semen.

Disclosure of Interest: None Declared

Keywords: Semen quality; boar fertility; CASA

Oral Abstracts - Wednesday 08 June 2016

O-REP2-007

Consequences of vaccination against gonadotropin-releasing factor on growth performance and reproductive development of heavy weight market gilts

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Introduction: Follicle development and consequently ovulation are influenced by a hormonal cascade initiated by gonadotropin releasing factor which stimulates secretion of luteinizing hormone through action on the ovary. Vivax (Zoetis, São Paulo, Brazil) triggers antibody production against GnRF, reducing the release of the naturally occurring gonadotropin hormone and leading to temporary involution of the reproductive organs. This regression is manifested through suppression of estrus in gilts. The aim of the present study was to assess the effects of vaccination against GnRF on feed intake, growth performance, and estrous activity in finishing gilts and to determine further consequences on reproductive tract development.
Materials and Methods: Gilts were initially weighed and allotted to a pen (n=72; 2 pigs/ pen) based on BW in a completely randomized design. Treatment group received the first anti-GnRF vaccine dose at 15 wk of age (V1) and the second dose at 19 wk of age (V2), control group received two injections of saline. Daily boar exposure (DBE) occurred from 21 to 25 wk of age, and the animals were slaughtered at 25 wk of age (S) (6 wks after second dose). Pen was the experimental unit for growth performance data. The percentage of all gilts that ovulated within treatments and the percentage of pens within each treatment that had one or both gilts ovulating during the trial were determined. Reproductive development was measured through ovarian and uterine weighing of 18 reproductive tracts within each treatment. Performance and reproductive tract data were analyzed by ANOVA (F-test) and estrus data by chi-square test.
Results: During the entire period (15 to 25 wk), BW, ADG and ADFI were 3.88 kg ($P< 0.05$), 60 g ($P < 0.05$) and 250 g ($P < 0.001$) greater in gilts immunized against GnRF (treatment group) compared with untreated gilts (control group), respectively. The differences in growth performance between treatments were mainly observed from V2 onwards. From 19 (V2) to 21 wk of age (before DBE begun), ADFI was 240 g greater ($P < 0.05$) and F:G ratio was 260 g greater ($P < 0.05$) comparing treated and control group. Between V2 and S vaccinated gilts had greater ADFI (+410 g; $P < 0.0001$) and ADG (+90 g; $P < 0.05$) and between DBE and S treated group showed greater ADG (+130 g; $P < 0.01$) and ADFI (+470 g; $P < 0.0001$). Ovarian and uterine weights were reduced ($P < 0.0001$) by 81.71 and 92.81%, respectively. Estrus was reduced by 82.06% ($P < 0.001$) in treated gilts compared with control gilts.
Conclusion: This data demonstrates that gilts immunized against GnRF were heavier, showed suppressed estrus, less developed reproductive organs, and improved daily gain and feed intake, primarily after second dose.

Disclosure of Interest: None Declared

Keywords: estrus suppression, Gilts, Vivax

O-REP2-208

Sows that fail to become pregnant show luteal regression at day 13 after mating.

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Introduction: In mated sows, corpus luteum (CL) function is important for the establishment of the pregnancy. The maximum CL size is established at day 8 - 9 of the pregnancy and maintained autonomous until day 12. Then, CL maintenance will depend on hormones such as LH and PGF2α. PGF2α is released from the endometrium at day 14 and triggers CL regression. This regression can be prevented in pregnant sows due to estradiol production from the attaching conceptuses; which decreases endocrine PGF2α release.
We hypothesized that a negative CL development between day 12 and 13 has a negative effect on the pregnancy rate in mated sows and on litter size in pregnant sows.
Materials and Methods: We performed a transrectal ultrasound examination (10 MHz, linear array probe, SV3513, Esaote SpA, Italy) of both ovaries and their CLs at day 10 (CLarea10) and 13 (CLarea13) after ovulation in 46 mated crossbred sows (Finnish Yorkshire x Finnish Landrace). The ultrasound images were analyzed on the computer using IMPAX 6.5.5 picture archiving and communication system (Agfa Healthcare, Belgium). We measured of each ovary the size (area in cm^2) of the five biggest CLs and averaged them. Furthermore, we calculated the difference of the average CL area between day 10 and 13 (CLarea13-10). Pregnancy detection was performed two weeks later and the numbers of alive, still born, and total born piglets were determined at subsequent parturition. We analyzed average CL area per pregnancy status using an independent two sample t-test and relations with litter size using a linear regression model (PASW Statistics v. 18.0.0).
Results: The parity was 3.8 ± 1 (mean ± SD), CLarea10 0.63 ± 0.11 cm^2, CLarea13 0.65 ± 0.13 cm^2, and CLarea13-10 0.02 ± 0.12 cm^2. At 4 weeks after insemination, 40 sows were detected pregnant (PREG) and 6 not pregnant (NONPREG). Pregnancy status at 4 weeks was not related with CLarea10 (PREG 0.62 ± 0.12 cm^2 vs. NONPREG 0.69 ± 0.07 cm^2), but was related with CLarea13 (PREG 0.66 ± 0.11 cm^2 vs. NONPREG 0.55 ± 0.18 cm^2; $P = 0.046$) and CL13-10 (PREG 0.05 ± 0.08 cm^2 vs. NONPREG -0.15 ± 0.16 cm^2; $P < 0.001$).No correlations were found between CLarea10, CLarea13, and CLarea13-10 and the number of total, alive, and still born piglets at subsequent farrowing.
Conclusion: The results indicate that there is no difference in the development of the corpus luteum between pregnant and not pregnant sows during the autonomous period. After that period, when the destination of the corpus luteum (CL) starts to be dependent on hormonal interactions, CLs of mated non-pregnant sows decrease in size, resulting in smaller CL at day 13 after ovulation. In pregnant sows, CL size at day 10-13 is not related with subsequent litter size.

Disclosure of Interest: None Declared

Keywords: corpus luteum, pregnancy

O-REP2-009

Relationship between ovulation rate and litter characteristics at birth

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Introduction: The genetic selection for increased litter size has resulted in a disproportionate increase in ovulation rate (OR). A higher OR was linearly related with decreased placental length at day 35 of pregnancy, which could inhibit foetal growth during further pregnancy (Da Silva et al., 2016). As increased litter size has resulted in a decreased piglet birth weight and increased within litter birth weight variation, we investigated the relationship between OR and litter characteristics at term.
Materials and Methods: Multiparous (parities 2-9) crossbred sows (Yorkshire x Landrace, n=109) were submitted to transrectal real time B-mode ultrasonography using an Aquila MyVet30 LAB with a convex transducer at 7.5 MHz (Pie Medical/Esaote, Maastricht, The Netherlands). Sows were scanned at day 24 ± 2.6 of pregnancy, and left and right ovaries were assessed for number of corpora lutea (CL), defined as OR, and the diameter (mm) of the five largest CL. At farrowing, the total number of piglets born (TNB, liveborn + stillborn), number of mummies and average piglet birth weight (BW) were assessed, and the standard deviation of piglet birth weight (SDBW) within the litter was calculated. The relationships of OR and CL diameter with litter characteristics were assessed in two different models with PROC MIXED in SAS 9.3 (SAS Inst. Inc. Cary, NC), and OR or CL diameter included as continuous fixed effect, and parity as fixed class effect.
Results: OR was 25.2 ± 3.8 (mean ± SD), ranging from 16 to 33 and the CL diameter 8.4 ± 0.8 mm. The number of mummies was 0.4 ± 0.9, TNB was 17.7 ± 3.1, BW was 1293 ± 188g and SDBW was 306 ± 76g. There was no relationship between OR and TNB (p=0.99), number of mummies (p=0.20), BW (p=0.30) or SDBW (p=0.66). However, a higher CL diameter was related with a higher BW (β=48.3 ± 22.9g/ovulation, p=0.04) and higher SDBW (β=25.0 ± 10.5g/ovulation, p=0.02).
Conclusion: The results show that OR did not affect litter characteristics at birth, despite previous reported effects on placental development at day 35 of pregnancy. Further, CL diameter is related with piglet BW and uniformity. Because CL diameter is related with the follicle diameter at ovulation, this could indicate that piglet development is already partly established during follicle and oocyte development. However, this needs further investigation.

Disclosure of Interest: None Declared

Keywords: Corpora lutea, ovulation rate, piglets birth weight

O-REP3-010

Risk factors for the different causes of piglet neonatal mortality in French farms

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Introduction: Neonatal mortality is one of the main issues of concern for the pig industry worldwide, resulting in decreased sow performance and significant economic loss. Although the issue of neonatal mortality has been explored previously, the variability in methodology makes it difficult to reach general conclusions. The relative proportions of each cause of death need to be identified. In this study we used a necropsy-based classification of the different causes of piglet mortality. We identified piglet mortality patterns and determined different farm profiles. Finally, we assessed the impact of different risk factors on specific causes of piglet mortality, in order to illustrate the absence of homogeneity in the issue of neonatal mortality.
Materials and Methods: A database of dead piglets, originating from 146 French pig farms between 2004 and 2014, was classified into 16 different categories of death according to an established protocol. The analysis was conducted first at farm level, to assess the correlations between the different causes of piglet mortality. A principal component analysis and a hierarchical clustering were used to identify neonatal mortality patterns. Subsequently, the analysis was conducted at piglet level on 7761 piglets. Risk factors were identified for the 6 main causes of piglet mortality and for all the other causes, grouped under the label “other causes”.
Results: Six main causes of mortality represented 84.5% of all the neonatal deaths. The percentage of deaths due to starvation and crushing were the only causes correlated with more than one cause of piglet death (R>0.30, p<0.05). Three patterns of neonatal mortality were identified, suggesting the existence of 3 recognizable farm profiles. Deaths during farrowing were significantly fewer during the night than during the day. The reverse was the case for deaths due to starvation, which were significantly lower during the day. A seasonal effect was suggested for the non-viable and the mummified piglets. The number of deaths per litter was significantly lower for these two causes. For the six main causes of neonatal mortality, the piglets which died from a specific cause tended to have more littermates which died from the same mortality cause. Parity, litter size and year also had significant effects on certain causes of death, such as crushing or the “other causes”.
Conclusion: Different patterns of neonatal mortality exist in French farms and relate to different risk factors. Further risk factors need to be identified in order to determine the optimal interventions (e.g. genetic vs management) for each farm profile.
This work was conducted under the EU-funded PROHEALTH project.

Disclosure of Interest: None Declared

Keywords: neonatal mortality patterns, Piglet mortality, Stillborn

Oral Abstracts - Wednesday 08 June 2016

O-REP3-011

Associations between hoof lesions and reproductive performance of sows in three Greek herds

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Introduction: Hoof lesions, which are very common in modern sows, were associated with high risk of early culling and compromised welfare of sows. Some hoof lesions were also associated with decreased litter weight, increased pre-weaning piglet mortality and higher odds of stillborn and crushed piglets. Evidently, if hoof lesions negatively affect not only sow longevity but also the important reproductive parameters which determine the utilisation of breeding herd's capacity and profitability of the farm, they should be on the target for improvement. In this study we investigated the associations between the severity of hoof lesions and three of the most important reproductive parameters of the sows, the number of liveborn and weaned piglets and the wean-to-first service interval, in three Greek farrow-to-finish herds.
Materials and Methods: All studied sows were individually housed during their previous gestations. Sows were examined for lesions on the sole, the heel, the white line, the wall and the coronary band and for toe and dew claw overgrowth, before farrowing. Data on the examined reproductive parameters was retrieved from the herds' productivity databases. Since, scoring of lesions on several hoof sites resulted in many correlated variables for each sow examined, we employed factor analysis to create a smaller set of uncorrelated variables (factors) which contained all the information of the original variables, and produced the corresponding factor scores. The number of liveborn and weaned piglets was associated with the produced factor scores in two multivariable linear regression models, whereas the possible association between the wean-to-first service interval and the factor scores was modelled with the use of zero-inflated negative binomial regression.
Results: The number of liveborn piglets was negatively associated with factor scores representing lesions on the heel ($P < 0.001$) and the sole of front feet ($P = 0.019$). The number of weaned piglets was also negatively associated with factor scores representing lesions on the heel ($P = 0.003$) of any foot, on the sole of front feet ($P = 0.001$) and on the white line, the sole and the wall of rear feet ($P = 0.008$), while the wean-to-first service interval was associated with factor scores representing lesions on the heel of any foot ($P = 0.02$), on the sole of front feet ($P = 0.02$) and of the dew claw length of front feet ($P = 0.009$).
Conclusion: Our results indicated that combinations of lesions on the dorsal and ventral part of the hooves, negatively affected the reproduction parameters considered, emphasizing the importance of general improvement of sows' hoof health.

Disclosure of Interest: None Declared

Keywords: hoof lesions, reproductive parameters, sows

O-REP3-012

Effect of using different amounts of diet in multiparous sows during the last third of gestation on body weights of piglets and in stillbirth number.

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Introduction: Feeding during gestation is relevant in order to maintain the body condition of the sow, support pregnancy and deliver the requirements for fetal growth, otherwise could affects reproductive performance and could impact body weight of the piglets at birth.
It is common that many producers during the last third of gestation increase the amount of feed in order to improve weight of piglets at birth and promote development of mammary gland. This has been well studied in nulliparous, but multiparous information is scarce.
The aim of this study was to evaluate the effect of using different amount of food (1 kg of difference) in multiparous sows with normal body condition (BC), from 85 days of gestation, on the body weight of live piglets born piglets and number of stillbirths.
Materials and Methods: The study was conducted at site 1 of a commercial farm, of 3800 sows approximately. Three hundred and thirty multiparous females, of at least one birth, normal BC, were divided into two study groups: a Control Group with 171 females, who received 3,2 kg/day of feed from day 85 of gestation, being identified with blue card; and a Treatment Group with 159 females that received 2,2 kg/day of feed from day 85 of gestation, identified with a red card.
The diet used in the farm, was based on corn, with 2984,97 kcal/kg ME for both groups.
At birth every piglet, of both groups, were weighed after the colostrum intake. Moreover, the numbers of stillbirths in both groups were recorded.
Statistical analysis was performed using ANOVA with Tukey test for the body weights of piglets and chi-square test for stillbirths, among groups.
Results: The total number of piglets born alive in the control group was 2464 with a body weight of 1346,18 gr (sd 685,32 gr), in addition, this group had 71 stillbirths. In contrast, the total pigs born alive in the treatment group was 2292 with a body weight of 1356,1 gr (sd 657,30 gr), in addition, this group had 64 stillbirths.
The statistical analysis do not shows significant difference in body weight of piglets born alive between groups (p=0,117) and no statistically significant differences in the number of stillbirths between groups (Chi square test: 0,0323, with p=0,857).
Conclusion: Based in the results of this study, no statistical differences in body weight of piglets born alive, as well as, the number of stillbirths among multiparous sows with normal BC that are feeding with 2,2 or 3,2 kg/day from the 85 days of gestation.
However, more studies are needed to assess the effect on body condition of post weaning sows, as well as the reproductive parameters that would have in the next gestation periods.

Disclosure of Interest: None Declared

Keywords: piglet weight, sow feeding

O-REP3-013

Effect of cross fostering on preweaning mortality

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Introduction: High mortality in the pig industry is a welfare problem and it reduces the farmer's income. The Danish Pig farmers have decided to reduce the mortality with 20% before 2020 compared to the 2011 level. For each percent the mortality is reduced in the farrowing units the gross margin per sow per year is increased by 6 euro. The objective of this study was to assess the impact of cross fostering and later movements of piglets on preweaning mortality.
Materials and Methods: The hypothesis was that cross fostering, later movements, and birth weight of pigs are associated with preweaning mortality.
The study was performed as a cohort study in 9 farrow-to-finish herds with more than 1.8 stillborn piglets per litter. Approximately 70 consecutive farrowings in each herd were included in the study. For sows their ID, parity, farrowing data, and treatments were recorded. Each piglet was weighed and ear tagged at birth. All pig treatments, cross fostering and later movements were recorded.
The statistical analysis was performed as survival analysis (Proc Phreg in SAS). Cross fostering was movements of pigs on the day of birth. Movements from day 2 were analyzed separately. Cross fostering and movements from day 2 were analyzed as time dependent variables. Only the first cross fostering on the day of birth and the first movement from day 2 was included in the analysis. Additional cross fostering or additional movements from day 2 were not included in the analysis. Birth weight was included as a covariate.
Results: A total of 8611 live born piglets were included in the statistical analysis and 1615 piglets died in the farrowing units (18.3%). On the day of birth 26% of the piglets were cross fostered. Between day 2 and day 20 30% of the pigs were moved. The hazard ratio for cross fostered pigs was 0.77 compared to not cross fostered pigs. This means that cross fostered pigs had approximately 23% lower risk dying before weaning. The hazard ratio for pigs moved from the second day was 2.49 compared to pigs that were not moved. This means that the moved pigs had approximately 2.5 times higher risk for dying before weaning. The hazard ratio for pigs with birth weights < 1 kg and 1.0-1.5 kg compared to pigs > 1.5 kg were 6.43 and 1.50, respectively.
Conclusion: This study indicates that cross fostering was associated with lower risk for preweaning mortality. Moving pigs from the second day and low birth weight was associated with higher risk for preweaning mortality.

Disclosure of Interest: None Declared
Keywords: Cross fostering, Preweaning mortality, Survival analysis

Oral Abstracts - Wednesday 08 June 2016

O-VET1-001

Staphylococcus aureus and MRSA colonization and infection in US swine veterinarians: an 18 month longitudinal study

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Introduction: Although research over 10 years has demonstrated a high risk of occupational exposure to MRSA in people working with pigs, the nature of nasal colonization with livestock associated MRSA (LA-MRSA) and its health implications remain poorly understood. We conducted an 18-month longitudinal study to understand patterns of S. aureus colonization of swine veterinarians.
Materials and Methods: Participants were given instructions for self-collection of nasal swabs and sent collection materials by mail. Participants were contacted monthly to collect a nasal swab and complete a survey to record recent pig exposure, events of physical injury focusing on skin wounds, and selected health events (skin or soft tissue infections, confirmed staphylococcal infections).
Results: Two subjects withdrew, but otherwise a compliance rate of 98% was achieved for swabs and surveys. Prevalence of *S. aureus* (64%) and MRSA (9%) in nasal swabs of veterinarians varied monthly from 58 to 82%, and from 6 to 15%, respectively. Culture positivity for individuals varied from 0% to 100%. The likelihood of positivity did not vary with interval from sample collection to processing, but decreased significantly with the interval between sampling and last pig contact. Predominant spa types were t034 (ST398, 50%), t002 (ST5, 25%) and t337 (ST9, 18%), which corresponds closely to isolates obtained in a concurrent study in pigs. Cocolonization with two spa types was observed in 71 positive samples, and some individuals were positive for all 3 MLST lineages at different times. Based on detection patterns, veterinarians were classified into three groups: Persistent carriers (PC, 36%), Intermittent carriers (IC, 62%) and Non-carriers (NC, 1%). Based on one-time quantitative testing of nasal swabs without enrichment, PC veterinarians carried significantly higher numbers of *S. aureus* than IC. Among PC veterinarians, the same spa type was detected at all sampling events in 9 PC veterinarians.
Conclusion: Elevated prevalence of S. aureus and MRSA in US veterinarians appears to be a consequence of exposure to pigs, however MRSA prevalence was much lower than seen in a similar study in Holland. Exposure did not lead to prolonged colonization in most subjects, and the higher numbers of S. aureus in PC subjects suggests that host factors may determine the likelihood of prolonged colonization by S. aureus of livestock origin. Although the period of follow up was limited, the absence of significant clinical infections despite regular exposure suggests that major health risks in healthy workers due to livestock associated S. aureus are unlikely.

Disclosure of Interest: None Declared

Keywords: MRSA, Staphylococcus aureus, Veterinarian

O-VET1-002

Administration of an acidifier (Selko-pH) via drinking water reduces shedding of Salmonella typhimurium in challenged piglets.

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Introduction: Salmonellosis is one of the most common and widely distributed foodborne diseases, with tens of millions of human cases occurring worldwide every year. Most cases of salmonellosis are mild, including diarrhea, fever, vomiting, and abdominal cramps; however, in some cases people die from salmonellosis. Pigs and pig meat are responsible for 15 to 23 % of all human cases of Salmonellosis in the EU – according to the European Food Safety Authority (EFSA). Controlling Salmonella more effectively within the pig meat food chain would have a direct impact on reducing the number of human cases. This project aims to develop dietary interventions to reduce Salmonella infection in pigs.
Materials and Methods: Twenty-four boar piglets were individually housed after weaning on tenderfoot slatted floors and divided over 3 treatment groups. One treatment group remained uninfected with Salmonella typhimurium (non-infected control group). The two other groups were orally infected with 1 mL Salmonella typhimurium at 9 log CFU/ml from 7 days after weaning for 7 consecutive days. One of the two infected treatment groups did not receive any treatments (infected control group), while the other infected treatment group received a blend of organic acids (Selko-pH, Selko BV, the Netherlands) in drinking water from weaning until the end of the study. Faecal shedding of Salmonella was quantitatively measured during three weeks by plate counts of faecal samples. Furthermore, body temperature and performance were recorded.
Results: Salmonella infection resulted in a slight fever in both Salmonella typhimurium infected groups (0.5 degrees increase of body temperature) and a peak Salmonella shedding of 5.49 log CFU/gram feces at 2 days after infection in the infected control group. During the week after infection a mild diarrhea and a reduction of body weight gain of 200 gram per day were observed in animals from the infected control group (182 gram per day in the Salmonella infected control group vs 382 gram per day in the non-infected control group). Selko-pH reduced peak Salmonella shedding by 1.44 log CFU/gram feces (4.05 log CFU/gram feces). In addition, body weight gain of animals receiving Selko-pH in drinking water was almost as high as that of uninfected animals (307 gram per day vs 382 gram per day).
Conclusion: Therefore; Selko-pH in drinking water seems to be a promising tool to control Salmonella in pigs.

Disclosure of Interest: None Declared

Keywords: Acidifier, pigs, Salmonella

O-VET1-003

The relationship between antimicrobial use, antimicrobial resistance and health and biosecurity status in Canadian grow-finish swine herds

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Introduction: Antimicrobial resistance (AMR) is a global threat to public health. The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) is a national integrated program dedicated to the collection, integration, analysis, and communication of trends in antimicrobial use (AMU) and AMR in selected bacteria from humans, animals, and food sources across Canada.
Materials and Methods: From approximately 95 sentinel grow-finish (GF) farms across the 5 major pork producing provinces, the CIPARS Farm Surveillance component actively collects pen fecal samples for susceptibility testing and data on farm demographics, animal health and AMU via questionnaires.
Results: In 2014, notable resistance detected in 147 *Salmonella* and 1672 generic *E.coli* isolates included: azithromycin 4%, 1%; ceftriaxone 4%, 2%; ampicillin 41%, 34%; and tetracycline 69%, 73% respectively. There were no *Salmonella* or *E. coli* isolates resistant to ciprofloxacin.
Overall (feed, water, and/or injection) the most commonly used antimicrobials in 2014 in the 95 enrolled herds included: penicillin G (59%), lincomycin (40%), tylosin (36%) and chlortetracycline (32%). Parenteral ceftiofur and florfenicol use was reported by 19% and 13% of farms respectively.
Antimicrobials were used in feed in 82%, by injection in 62%, and in water in 28% of GF herds. In 9% of herds no AMU was reported. There were an estimated 83 mg of tetracyclines, 33 mg of macrolides, and 28 mg of lincosamides consumed in feed during the GF period after adjusting for population and pig weight (Total mg of antimicrobials consumed through feed / (Total number of pigs in the sampled GF periods x ESVAC standard weight of 65 kg).
Reported use of antimicrobials was most common for *Streptococcus suis* and *Lawsonia*. The number of diseases reported on each farm ranged from 0 to 11 with a median of 5. Boots, coveralls and a biosecurity sign were the most commonly reported biosecurity measures.
Preliminary modelling results showed significant factors associated with AMU in GF herds included: number of diseases, PRRS status, and region (increased use) and *Mycoplasma* status of the sow herd and *Lawsonia* vaccination in the nursery (decreased use). Significant factors associated with ceftiofur use in GF herds included: *E.coli* and *Salmonella* status, multiple sources of pigs, and region (increased use) and number of diseases (decreased use). Further modelling will be conducted to examine additional relationships between disease pressure, biosecurity measures, AMU and AMR.
Conclusion: Significant relationships exist between GF AMU and demographic factors, specific diseases, and vaccinations and can include earlier stages of production.

Disclosure of Interest: None Declared

Keywords: antimicrobial resistance, antimicrobial use, grower-finisher pigs

O-VET1-004

Image analysis for automated detection of abnormal organs in pig offal

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Introduction: Visual inspection of carcasses is an important means of ensuring the safety and quality of meat products, enabling the detection of pathological conditions and public health hazards. It may also generate data on subclinical diseases that can be provided to pig producers. However, manual inspection is labour intensive, subjective and limits detailed screening for the purposes of health schemes. In addition, recent EU legislation introduced changes to minimise handling of carcasses and offal at abattoir on safety grounds. These factors motivate the need for the development of automated meat inspection systems based on image analysis.
Materials and Methods: We used 350 high-resolution colour images acquired at abattoir, each showing a group of non-digestive track organs (pluck), including the heart, lungs, diaphragm, and liver. All images were annotated by a specialist veterinarian for the presence of signs of pathologies, namely: pericarditis, enzootic pneumonia, pleuropneumonia, pleurisy, lung abscesses, liver milk-spots, and peritonitis. These annotations enabled the use of machine learning to train image analysis algorithms designed to: detect the pluck; localise individual organs within the pluck; and classify each organ as 'normal' or 'abnormal'. This implies that 'abnormal' organs contained pathologies, but there was not distinction between them. The algorithms were combined to yield diagnostic tests for abnormal organs.
Results: The test for lung abnormality had sensitivity 0.52 and specificity 0.84. The test for liver abnormality had sensitivity 0.84 and specificity 0.66. These initial results are based on a small amount of training data. Our data set of annotated images is continuously growing and, by the time of IPVS 2016, we expect to have substantially improved results to report, not only for the lungs and liver, but also for the heart and diaphragm.
Conclusion: Automated visual inspection can help to prevent public health hazards, provide feedback to farmers, and minimise carcass handling at abattoir. We developed a system prototype to detect abnormal organs in pig offal. Preliminary results reveal high specificity in testing for abnormal lungs, and high sensitivity in testing for abnormal livers.

This project was co-funded by Innovate UK and the Biotechnology and Biological Sciences Research Council (BBSRC).

Disclosure of Interest: None Declared

Keywords: Automated inspection, Image analysis, Pig offal

Oral Abstracts - Wednesday 08 June 2016

O-VET1-005

Effect of strategic administration of sodium butyrate in the late finishing period on Salmonella control

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Introduction: On-farm control measures may provide a useful element in reducing *Salmonella* carriage in pigs, thereby potentially improving food safety. This study investigated the effectiveness of strategic administration of sodium butyrate for ~4-weeks prior to slaughter as a low-cost means of controlling *Salmonella* shedding in finisher pigs on two highly contaminated farms.
Materials and Methods: Two trials (A and B) were conducted on two commercial pig farms, both with a history of high *Salmonella* seroprevalence. In both trials, pens (14 pens of 12 pigs/pen in Trial A and 12 pens of 12-17 pigs/pen in Trial B) were randomly assigned to a control (finisher feed without sodium butyrate) or treatment group (the same feed with 3 kg sodium butyrate/tonne) for 24-28 days. Faecal samples were collected from each pig on days 0, 12 and 24/28. Pigs were tracked to the slaughterhouse where blood, caecal digesta and ileocaecal/mesenteric lymph nodes were collected. Pigs were weighed at the start and end of the treatment period, feed intake was measured weekly, and carcass quality parameters were recorded at slaughter.
Results: : In Trial A, *Salmonella* shedding was reduced in the sodium butyrate-treated group compared to the control group at the end of the treatment period (30% versus 57% probability of detecting *Salmonella* in faeces, respectively; $p<0.001$). This was reflected in the serology results, with lower seroprevalence found in the treated group compared to the control group using the 20% optical density cut-off (69.5% versus 89%; $p=0.001$). However, no effect of treatment was observed in Trial B, in terms of faecal shedding or seroprevalance, which could perhaps be explained by detection of a concomitant infection with *Lawsonia intracellularis*. Significant differences in *Salmonella* recovery rates were not detected in the caecal digesta or lymph nodes in either trial. Furthermore, feed intake, weight gain, and feed conversion efficiency (FCE) did not differ between treatment groups ($p>0.05$) in either trial. However, numerical improvements in weight gain and FCE were found with sodium butyrate treatment, which gave a cost benefit of €0.04/kg of live-weight gain in Trial A.
Conclusion: Overall, results suggest that strategic feeding of sodium butyrate to finishing pigs for 24-28 days prior to slaughter can reduce *Salmonella* shedding on-farm and seroprevalance, although it did not impact *Salmonella* carriage in intestinal samples taken at slaughter. Growth performance was not significantly improved but there was an economic benefit associated with sodium butyrate administration.
Disclosure of Interest: None Declared
Keywords: Control, Organic acids, Salmonella

ANTIMICROBIAL

O-VET2-006

Is there an association between the use of antimicrobials in pigs and resistance levels in commensal E.coli after a period of major reduction in use?

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Introduction: Worldwide there is growing concern about the increase of antimicrobial resistance. Antimicrobial resistance (AMR) could be considered as the result of (over)usage of antimicrobials (AMs) in human beings and animals. In order to reduce further increase of AMR the government of the Netherlands together with the livestock sectors and vets developed an action plan to reduce the use of AMs, in the assumption that reduction is associated with reduction in resistance. The plan was focused on the reduction of AMs. Transparency in the use of AM's, creating awareness of the risks of AM use, improvement of the health status on pigfarms, and goals set by the government were part of this action plan. After a period of major reduction in the use of AMs, the question can be asked if there is an association between the use of AMs in pigs and resistance levels in commensal *E.coli*?
Materials and Methods: Data of the use of AMs were obtained from the Agricultural Economic Institute (2004 to 2012) and from data published by the Netherlands Veterinary Medicines Authority (SDa, 2011 -2014). Data of AMR were obtained from the Central Veterinary Institute as published in MARAN. Data of usage were given as defined daily dosage per animal per year (DDDAY), the data of the CVI were presented in percentages based on MIC's interpreted using epidemiological cut-off values. Ten antibiotics were tested. Logistic regression analysis for grouped data (number of resistant isolates over the total tested) was used to obtain OR for *E.coli* isolated be resistant to each antimicrobial agent per 1 unit increase in total DDDA/Y or homologous use.
Results: AM use increased from 2004 till 2009 and decreased thereafter with 53.6% (11 DDDA/Y) whereas the percentage of total AMR slowly increased till 2009 and decreased afterwards with 21.6%. Moreover restriction of the use of 3rd/4rd generation cephalosporins and fluorochinolons resulted in 86% and 100% reduction of cefatoxime and ciprafloxic resistance respectively. Total AMuse was positively related to resistance. One unit increase in DDDA/Y was associated to a ~8% increased odds of total and most AMs and up to 30-40% increased probability of ciprofolxacin ans nalidixic acid resistance.
Conclusion: The Dutch policy to set goals for the reduction in use, together with the measures taken by vets and farmers and the establishment of an independent authority (SDa) to analyze the data of the use of AMS at farm level, increased the awareness of vets and farmers of AMR and resulted in a major reduction in the use of AMs. The decrease in the use of AMs is associated with the decrease in AMR. Reduction in use has been proven an efficient way to reduce antimicrobial resistance in farm animals
Disclosure of Interest: None Declared
Keywords: antimicrobial resistance, antimicrobial usage

ANTIMICROBIAL

O-VET2-007

Evolution of antimicrobial use between 2010 and 2013 in farms of the INAPORC Panel and analysis of treatment patterns by animal category

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Introduction: The French pork industry has been strongly involved for several years in a process of reducing antimicrobial use in pigs. In France, two complementary tools quantify the evolution of antimicrobial use. Anses-ANMV monitors annual sales of antimicrobials and has observed a decrease in exposure of pigs by 22 % between 2010 and 2013. The "INAPORC Panel" quantifies antimicrobial use and records treatment patterns in a representative sample of pig farms on a regular basis. This study aims to assess the evolution of antimicrobial use over 2010-2013, detailing animal categories and reasons for treatment.

Materials and Methods: A representative sample of pig farms was randomly selected in the National Swine Database of Identification (BDPORC). Inventory of antimicrobials bought by farmers in 2013 was collected from the veterinarians and medicated feed manufacturers. During a phone call, farmers reported for each product the categories of animals treated and reasons for treatment. Indicators used to express the results were the number of Animal Daily Dose per animal produced (ADD/animal) and the relative frequency of farms concerned by each kind of treatment (pharmaceutical form, antimicrobial family, reason for treatment). Comparisons between 2010 and 2013 were subsequently performed.

Results: Thanks to the high participation rate of farmers (75 %), the study involved a representative sample of 157 farms. Over three years, the average number of days of treatment in suckling piglets and weaned piglets significantly decreased by 29 % and 19 % respectively. In fattening pigs, the drop reached 29 % (not significant). Several reasons contributed to explain these decreases: the publication of the French Ecoantibio2017 Plan, the implementation of many awareness actions on antimicrobial resistance, the increasing restrictions on deliveries of medicated feed and the better targeting of treatments administered by dosing pumps. Cephalosporin usage has also decreased by 90 % in suckling piglets further to the decision taken in 2010 by the French pig sector and the veterinarians to voluntarily limit their use. In weaned piglets, antimicrobial treatments for digestive problems still dominated in 2013 (62 % of their ADD/animal) although they decreased by 29 % since 2010. Only antimicrobial use in sows increased by 17 % (not significant), which is probably related to the group-housing of pregnant sows, associated with more locomotor and urogenital problems.

Conclusion: This simple and accurate professional tool helps to provide reliable and detailed baseline data on antimicrobial use in pig farms. The results reflect the efforts made by the French pork industry to decrease antimicrobial use.

Disclosure of Interest: None Declared

Keywords: animal daily dose, antimicrobial usage, treatment patterns

ANTIMICROBIAL

O-VET2-008

The impact of vaccination on the consumption of antimicrobials in Danish pig herds, 2013

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Introduction: Antimicrobial agents are being used extensively in modern swine production worldwide causing concern for the development of antimicrobial resistance. Identification of efficient alternatives to routinely applied antimicrobials has therefore become a subject of interest. We explored the impact of routinely used vaccination as an alternative to antimicrobial consumption in ordinary pig herds.

Materials and Methods: It was hypothesised that herds with increased use of vaccination would have a lower antimicrobial consumption than herds not using vaccination. Data were obtained from VetStat (the Danish database with prescriptions of medication for livestock use) and the Danish Central Husbandry Register. All one-site system pig herds, active in year 2013, with >50 sows and >200 weaners were selected for the study. The analysis included three different vaccines against Porcine Circovirus Type 2 (PCV2), *Mycoplasma hyopneumoniae* (M_HYO) and *Lawsonia intracellularis* (LAW), as well as herd size. Data were analysed using first univariable models taking into account one vaccine at a time. Secondly, a multivariable linear regression model was used taking into account all three vaccines simultaneously. The outcome was antimicrobial consumption in weaners for all models.

Results: Out of the 1,513 herds selected for the study, 1,415 herds had antimicrobials prescribed for gastrointestinal disorders, and 836 for respiratory disorders. PCV2 vaccine was used in 880 herds, M_HYO vaccine in 787 and LAW vaccine was the least used, with 115 herds using it. Herds using PCV2 vaccination had higher antimicrobial consumption overall, and with gastrointestinal indication, than herds not using the vaccine. Herds using M_HYO vaccination had higher antimicrobial consumption in general, and with respiratory indication, than herds not using the vaccine. Concerning PCV2 and M_HYO vaccination, the same tendency of results were observed in multivariable analyses. Herds using LAW vaccination had a lower antimicrobial consumption with respiratory indication, compared to the herds that did not use the vaccine. This suggested that antimicrobials could have been used for other disease categories than those officially prescribed by the veterinarians. These results were no longer statistically significant in the multivariable analyses.

Conclusion: On average, herds using the different combinations of vaccines had higher use of antimicrobials than herds not using the vaccines – probably as a result of health problems in the herds existing prior to the initiation of vaccination or concurrent disease.

Disclosure of Interest: None Declared

Keywords: alternatives, antimicrobial consumption, vaccination

Oral Abstracts - Wednesday 08 June 2016

ANTIMICROBIAL

O-VET2-009

Antimicrobial use and biosecurity practices in pig production in four European countries, comparison, associations and opportunities.

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Introduction: The global development and spread of antimicrobial resistance is largely driven by the extensive use of antimicrobials both in human and animal medicine. Within livestock production, pig husbandry has been identified as one of the main consumers of antimicrobials. To allow for the development of plans for antimicrobial usages reduction, first a detailed understanding of the current use and its associations with animal health and production characteristics is required.

Materials and Methods: We studied the antimicrobial use in 227 pig herds originating from 4 European countries (Belgium, France, Germany, Sweden) and its association with animal health, production, biosecurity and other disease prevention measures. Each farm was visited once to collect detailed information on the antimicrobial consumption, internal and external biosecurity, technical performances, health characteristics and vaccination practices. A causal path was designed to study associations between biosecurity status, antimicrobial usage, and production parameters.

Results: Huge differences in antimicrobial use were observed within and between countries with an average treatment incidence from birth till slaughter of 243 in Germany (42 for the sows), in Belgium 143 (16 for sows), in France 108 (22 for sows) and in Sweden 23 (11 for sows). These results indicate that an average fattener was treated 10 times more with antimicrobials in Germany than in Sweden. As the biosecurity is concerned, Sweden had on average the highest total biosecurity status (63.7), followed by Germany (63.0), France (58.6) and Belgium (57.8) In all countries external biosecurity scored higher compared to internal biosecurity.

It was found that antimicrobial usage in sows was significantly associated with the antimicrobial usage from birth till slaughter in the growers, the use of anti-inflammatory products in weaners and the number of pathogens vaccinated against, suggesting an overall higher disease pressure. Higher antimicrobial usage from birth till slaughter was associated with a shorter farrowing rhythm and a younger weaning age, whereas a better external biosecurity was related with a lower antimicrobial usage. A higher external biosecurity was associated with more weaned piglets per sow per year.

Conclusion: Overall the study showed high variation both in antimicrobial usage, biosecurity and management practices indicating substantial room for improvement. Moreover some cross country associations were identified allowing for potential interventions such as using a longer farrowing rhythm, weaning of the piglets at an older age and improvement of the biosecurity status.

Disclosure of Interest: None Declared

Keywords: antimicrobial consumption, biosecurity, Production Performance

SALMONELLA

O-VET3-010

Growth depression of fattening pigs in relation to individual Salmonella serology

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Introduction: Serological Salmonella status of fattening farms is becoming more important in the Netherlands. Several slaughter houses don't accept Salmonella status III pigs any more under regular terms. The objective of this study was to look at individual OD% values after natural infection and to determine if individual growth of pigs is related to individual Salmonella OD%. If so, investments to improve Salmonella status can be compensated by better growth.

Materials and Methods: PCV2 vaccinated (Topigs 20 x Pietrain) piglets arrived in February 2015 at the Vlierbos (trial farm of de Heus Feed Company in the Netherlands) in one batch for a feed trial. For this trial cleaning and disinfection of the pens was performed in a poor way. As a part of this trial, serology for Salmonella was performed. The farm is equipped with 72 pens each containing 9 or 10 animals. A total of 37 gilts (1 per pen) was randomly selected at arrival at 10 weeks of age. The selected gilts (mean weight at arrival about 20 kg) were individually weighted and blood was taken at the age of 10, 15, 20 and 25 weeks of age. Salmonella OD% were determined by using a Salmonella mix-Elisa. OD% >= 10 was considered positive. Four animals with outliers in data for ELISA OD% and bodyweight (BW) were removed. Significance was calculated using SPSS ANOVA test.

Results: At 10 weeks old all Salmonella OD% were < 10 except 3 animals with OD% of 13, 16 and 63. OD%>values increased quickly in almost all pens after start of the trial. At 15 weeks old, the mean OD% was 43 (with 25 of 33 pigs positive), at 20 weeks 55 and at 25 weeks 35.

Small piglets had the highest OD% at 15-weeks; 17 pigs < 20 kg BW at 10 weeks of age had a mean OD% of 61.5 at 15 weeks of age (8 of them >= 60). 16 pigs >= 20 kg BW at 10 weeks of age had a mean OD% of 23.2 at 15 weeks of age (P =0.002) (1 of them >= 60).

Growth between 10 – 25 weeks old was 80.1 kg for 24 pigs with OD%< 60 at 15-weeks of age. Growth for 9 pigs with OD%>= 60 in week 15 was 73,4 kg (P=0.007). Eight of these 9 pigs with high OD% had a BW < 20 kg at the age of 10 weeks.

Pigs with high maximum (>=80) OD% show the largest decrease in OD% during the 5 and 10 weeks after an animal reached the maximum OD%. These animals had an OD%>40 at the end of the trial.

Conclusion: In this trial Salmonella infections were active in the first weeks after the trial started. Especially the smallest pigs at the start of the trial had the highest individual Salmonella OD%>values and lowest 10-25 weeks growth. It's not clear whether growth depression is a result of Salmonella infection, a lower BW at the start of the trial or a combination of these factors. High maximum OD%>values show the fastest decrease.

Disclosure of Interest: None Declared

Keywords: body weight, Growth depression, individual Salmonella serology

SALMONELLA

O-VET3-011

Investigation of in-feed organic acids as a low cost strategy to combat Salmonella in weaned pigs

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Introduction: *Salmonella* carriage in pigs is a significant food safety issue, with 8.9% of European human cases of salmonellosis linked to the consumption of pork or pork products. In terms of cost to the producer, *Salmonella* control measures implemented during the early stages of production are highly desirable. The inclusion of organic acids in the diet has previously been shown to reduce shedding and transmission of *Salmonella*. The aim of the present study was to examine the effect of three commercially available organic acid products on *Salmonella* levels in weaned pigs, using a model of experimental infection that closely mimics natural exposure to the organism.
Materials and Methods: Trial pigs (n=40) were placed in one of four *Salmonella* contaminated pens with 10 pigs per pen. Pens had previously been contaminated to a level of 10^3-10^4 CFU/g of faeces by housing two pigs experimentally challenged with 5 x 10^6 CFU of a monophasic variant of *Salmonella* Typhimurium in the pens for 5 days. The challenged pigs were then removed from the pens and euthanised. Trial pigs received one of four diets: T1, control diet; T2, sodium butyrate supplemented diet (3Kg/tonne); T3, benzoic acid supplemented diet (5Kg/tonne) and T4, formic/citric acid supplemented diet (4Kg/tonne). A further 10 pigs were placed in a *Salmonella*-free pen receiving T1. Pigs were weighed and blood sampled on Days 0 and 28. Faeces was collected on Day 0, 2, 3, 5, 7, 14, 21 and 28 and examined for the presence and quantity of *Salmonella*. On day 28, five pigs per group were euthanised and ileocaecal lymph nodes and caecal contents sampled for culture. The remaining five pigs per pen were then fed T1. Faeces was collected from the remaining pigs on Days 35 and 42. On day 42 pigs were weighed, euthanised and ileocaecal lymph nodes and caecal contents tested for *Salmonella* levels. The trial was replicated once.
Results: 96% of pigs were infected within the first 2 days of exposure to the contaminated environment. Most pigs shed *Salmonella* at levels of between 10^0 and 10^4 CFU/g faeces for at least 7 days post-exposure. The prevalence and concentrations of *Salmonella* decreased in all groups between days 7 and 28. Negative control pigs remained *Salmonella*-negative throughout the trial. Preliminary analysis shows that there were no significant differences in *Salmonella* shedding between the pigs fed the control diet and those fed the treatment diets.
Conclusion: Results suggest that the organic acids used in this study failed to prevent infection or reduce *Salmonella* shedding. Further analysis is on-going to determine the effects of the acids on growth performance of the pigs.

Disclosure of Interest: None Declared

Keywords: Organic acids, Pigs, Salmonella

SALMONELLA

O-VET3-012

Salmonella spp. infection in piglets from Salmonella-positive breeding holdings

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Introduction: The dynamic of pig salmonellosis during the first weeks of life is barely known. Studies suggest that *Salmonella* prevalence is very low as the percentage of shedders is low (<9%), but they are usually based on analyses of a small amount of fecal matter, which yield poor diagnostic sensitivity. Thus the true prevalence of infection is unknown, and therefore the potential of these young pigs to become shedders. This study shows results of a project aimed at assessing the level of infection in piglets from *Salmonella*-positive sow farms.
Materials and Methods: A total of 405 four weeks-old (wo) and 334 six wo pigs from 5 farms were included in this study. The maximum possible amount of mesenteric lymph nodes (MLN) and fecal content (FC) was collected at slaughter for bacteriology (EN ISO 6579:2002/A1:2007) and serotyping performed on positive samples. Diaphragm juice was used for serology (HerdCheck® Swine *Salmonella* ELISA).
Results: A total of 247 (33.4%) MLN-positive (i.e. infected) and 251 (33.9%) FC-positive (i.e. shedding) piglets were identified. Both proportions were higher for 6-wo pigs compared to 4-wo pigs (38.6% *vs.* 29.1%, and 39.2% *vs.* 29.6%, respectively; $P<0.01$). Prevalence of infection was higher in summer (57.7% *vs.* 28.6% in autumn, 30.2% in winter or 25.3% in spring). About 67% of the infected pigs shed the pathogen. Overall, the odds of shedding *Salmonella* for an infected piglet was 10 times higher than that for a non-infected one (OR=9.8; 95%CI:6.8-14.2).
Out of 260 isolates serotyped, Rissen was the most common serotype (38.8%), followed by 4,[5],12:i-: (23.5%), Typhimurium (14.2%), Brandenburg (7.3%), Goldcoast (7.3%), Derby (5.4%). A distribution similar to that found for sows from the same farms (data not shown).
Out of 659 piglets serologically analyzed 34% were seropositive. Seroprevalence was higher for 4-wo pigs than for 6-wo pigs (45.6% *vs.* 20.8%; $P<0.01$). The odds of being *Salmonella* infected was lower for seropositive pigs (OR= 0.63; 95%CI: 0.4-0.9).
Conclusion: The prevalence of *Salmonella* infection and shedding among piglets was high, which could have been put in evidence due to the thorough analyses done. The serotypes identified suggested a sow-to-piglet transmission. Infection was also associated with seasonality. All results together suggested that better hygiene and isolation of farrowing units should help to reduce infection.
Seropositivity decreased as age increased, and it was associated with a lower proportion of infected piglets, suggesting the presence of maternal antibodies that would confer some protection. Boosting humoral protection through sow/piglet vaccination and assuring proper colostrum intake may help to reduce infection levels.

Disclosure of Interest: None Declared

Keywords: epidemiology, piglets, salmonellosis

Oral Abstracts - Wednesday 08 June 2016

SALMONELLA

O-VET3-013

A longitudinal study on Salmonella shedding in naturally-infected pigs through the grower stage until slaughter

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Introduction: The presence of multi-drug resistant *Salmonella* spp. in the swine population is an important food safety concern. Understanding the distribution and patterns of *Salmonella* shedding from the grower stage until slaughter will aid to improve prevention and control strategies. The objectives of this study was to determine how long pigs shed *Salmonella* if they enter a grower barn already naturally-infected and whether *Salmonella* can be detected from such pigs at slaughter.
Materials and Methods: Naturally-infected 9-week-old pigs (n=45) were purchased from a farm with a history of *Salmonella* infection. The pigs were housed at the Ponsonby Animal Research Facility, at the University of Guelph. Individual weekly fecal samples were collected. When pigs reached market weight, they were slaughtered (~86.8kg; average carcass weight) at the University of Guelph abattoir. Tissue samples (ileocecal lymph node, neck lymph node, spleen, liver, tonsils) and cecal content samples were collected from each pig. Fecal and tissue samples were cultured for *Salmonella* using Tetrathionate broth (enrichment method) and XLT4 (agar plate). Agglutination test was used to confirm *Salmonella*.
Results: No clinical signs of salmonellosis were observed. Over the 10 weeks of sample collection all pigs tested positive for *Salmonella* shedding at least once and 89% of pigs more than once, with one pig testing positive 8 times. Out of the 45 pigs, 41 pigs were positive 4 times or less and 4 pigs tested positive 5 times or more. Prevalence of the *Salmonella* shedding was 80% and 91% in Week 1 and Week 3. During week 3, 2 pigs were culled due to rectal prolapse. *Salmonella* shedding rate was 33%, 9%, 12%, 9%, 7%, 5%, 2%, and 5% at Week 4, 5, 6, 7, 8, 9, 10, and 11 (age of 20 weeks), respectively. At slaughter, *Salmonella* was isolated from 7 pigs (16.3%; 7/43). *Salmonella* was found in one or more of the following tissues: ileocecal lymph node, neck lymph node, spleen, liver, or tonsil, and three times in cecal contents. Of 7 pigs harboring *Salmonella* at slaughter, 5 (71%) of those had not tested positive on weekly fecal checks for at least 7 weeks or longer.
Conclusion: This study found that asymptomatic carriers could become chronic shedders of *Salmonella* for up to 8 weeks in the grower phase. The absence of *Salmonella* detection in fecal samples in pigs in the late finisher stage is no indication that *Salmonella* will not be found in tissues at the time of slaughter, posing a food safety concern.
Acknowledgments: Financial support from OMAFRA-University of Guelph Research Partnership, OMAFRA Food Safety Research Program, Swine Innovation Porc, & Huvepharma

Disclosure of Interest: None Declared

Keywords: Food Safety, Salmonellosis, Swine

O-RES-001

Case report: Leg lameness in gilts

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Introduction: In two belgian farrow-to-finish herds problems of leg weakness occurred in gilts. Danish gilts arrived in Belgium at 20kg, stayed in quarantine in a rearing unit and were transported to herd 1 at 180-240 days of age. They were fed a rearing diet *ad lib*. Between 8 and 10 days after arrival at the herd almost 50% of the gilts started limping. Only the hind limbs were affected, with discrete swelling of the joints. Gilts purchased from different breeding units in Denmark were directly transported to herd 2, where they were kept in quarantine during 15 weeks. The animals were fed a rearing diet twice a day. Gilts were supplemented with 50g monocalciumphosphate on a weekly basis until farrowing. More than 80% of the gilts showed swelling of the joints, with variable degrees of lameness, starting from 1 week after arrival.
Materials and Methods: The differential diagnosis of leg weakness in gilts embraces trauma, OCD, deficits in Ca en bacterial infections. Deficits in Ca can be caused by nutritional imbalance. The gilts' quarantine diets were analysed and serological analyses were done of the gilts. Necropsies were performed on two gilts, together with histological and bacteriological examination.
Results: No remarkable deficits were discovered in the diets. Serological analyses showed a normal Ca concentration. However, concentration of P was too high. Serological Ca/P ratios of >4 have been found. Concentrations of osteocalcin were too low (<11µg/l), indicative for bad bone turnover or insufficient bone formation. In addition, CTx, a marker of bone mobilization, was too low. Necropsies demonstrated discrete injuries at the cartilage at the femur heads. Both knee joints were filled with hemorrhagic fluid and mild cartilage injuries were present on the condyles. Histological examination revealed distinct hyperplasia and hypertrophia of the synoviae and perivascular infiltration of round cells, being an image of subacute infectious arthritis. Bacteriological examination showed a positive PCR for *M. hyosynoviae.*
Conclusion: The purchased gilts were probably carriers of *M. hyosynoviae.* During transport to the herds, they were exposed to stress. This caused a penetration of the bacteria in the blood stream, moving to the joints, resulting in discrete arthritis, swelling of the joints and pain, which resulted in limping gilts 1 to 2 weeks after arrival. Treatment with high dose antibiotics (macrolides and spectinomycines) and NSAIDs solved the acute problem. To avoid similar problems in the future, preventive measures mainly emphasise avoiding stress (stocking density, housing conditions, transport, etc.). Besides, precaution needs to be taken considering nutritional imbalance and (viral) co-infections.

Disclosure of Interest: None Declared

Keywords: diagnosis, Gilts, Mycoplasma hyosynoviae

Residents' ECPHM Session

O-RES-002

Follicular dynamic and hormonal pattern in weaned sows with and without ovulation synchronization with GnRH analogue. Effect on single fixed time AI

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Introduction: To be reproductively efficient is a challenge in the modern swine production systems. Fixed time insemination not only reduces costs using less doses of semen but also allows using boars of better quality, reducing the variability and improving the quality of the resulting offspring. This study aimed to understand the follicular dynamics and hormonal pattern in two groups of sows inseminated at a single fixed time.
Materials and Methods: 18 weaned sows were randomly splited in two groups. Treated sows were administered 10µg of buserelin (GnRH analoge), 85 hours after weaning (n=9) vs non-treated Control group (n=9). Both groups were scanned from 72 hours after weaning to ovulation every 8 hours, the size of three follicles were measured every scanning. The ovulation was considered happened 4 hours before of the presence of corpora hemorragicae (CH) with or without follicles. Every 8 hours an evaluation of the oestrus were done with a mature teaser boar from 72 hours after weaning to the end of oestrus. Blood samples were taken to evaluate the estrogen (from 72 hours of weaning to 24 hours after ovulation) and progesterone (day 0, 1 and 10 after artificial insemination). All sows were artificially inseminated (AI) with a single fixed time AI with semen from the same ejaculate, 118 hours after weaning.
Results: Weaning to ovulation interval (WOvI) was 118±8.94 and 116±3.52 h for the Control and GnRH groups, respectively ($p<0.05$). Follicular size had differences 24 h after treatment (6.42±0.38 vs 7.04±0.39 mm for Control and GnRH groups, respectively, $p<0.05$). All the sows were pregnant at 26 days after insemination. At that time, the ultrasonography showed differences ($p=0,07$) in number of embryonic vesicles (11±2.64 vs 14±18.11 for Control and GnRH groups, respectively). No significant differences were observed in oestrus length, interval between oestrus and ovulation, follicular size and estrogen serum concentration (pg/ml) at the moment of the ovulation, were observed between both groups.
Conclusion: The use of GnRH analogue was effective on ovulation synchronization in weaned sows, showing also higher size of the follicles just before ovulation for most of the treated sows. The concentration of estrogens seems to be independent of the follicular size although there were statistically significant differences in the follicular size. It is very likely that a single fixed time for AI needs ovulation synchronization for good results in terms of profitability, since it increases the number of embryonic vesicles.

Disclosure of Interest: None Declared

Keywords: follicular dynamic, GnRH analogue, Single fixed time IA

Oral Abstracts - Wednesday 08 June 2016

Residents' ECPHM Session

O-RES-003

Water quality: differences of perception and management between poultry and pig producers

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Introduction: Drinking water is an essential nutrient for animals. Indeed, when the physiological animal's requirements are not satisfied, performances can decrease and/or diseases may appear, both having an economical impact for pig or poultry productions. On field, waterlines cleaning protocols seem more frequent in poultry farms than in pig farms. Is it a reality? Are poultry farmers more aware of water quality than pig producers?
In order to update data concerning the different perception and practices for water management in pig and poultry farms, a study has been conducted to compare water supplies, their optimization and the different management practices for piglets in post weaning rooms and broiler chicken.
Materials and Methods: A survey was carried out in pig and poultry farms located in the West region in France. Twenty-five pig producers and 25 poultry farmers have been selected. A questionnaire has been filled during an interview with farmers. The association between practices and the characteristics of the production has been analyzed with Khi-deux tests.
Results: In all the interviews, pig and poultry farmers state that water quality is a main concern. Both bacteriological and chemical parameters are regarded as important for water quality, even if chemical analysis is less frequently performed. Water mainly comes from drilling (50 % for both productions). Sixty per cent of pig farmers use continuous water disinfection (chlorination) whereas 80 % of poultry farmers perform it with different disinfectants (the remaining 20 % use tap water). Water is also an administration route for antibiotics, anthelmintics, vaccines and nutritional factors. Regarding sanitary status of animals, some recurrent diseases can be linked to unadapt water quality. Digestive disorders during the post-weaning period (82 %) and lameness of birds (90 %) are the most frequent disorders.
For many criteria poultry farmers are more aware of water quality than pig farmers. The main differences in their practices concern the monitoring of water consumption and the water pipe maintenance (including cleaning measures to eliminate the biofilm). None pig farmers perform pipes' draining while 72 % of poultry farmers do. During the down period, all the poultry farmers set up protocols with mechanical and chemical procedures: 44 % use flushing, draining, base, acid and disinfectant whereas 24 out of 25 pig farmers only clean the troughs in post-weaning rooms.
Conclusion: This study underlined that the control of water management is more settled in poultry farming compared to pig industry. These different treatments and maintenance practices could help to prevent digestive disorders in weaners and thus to reduce antibiotic consumption.

Disclosure of Interest: None Declared

Keywords: pig farm, poultry farm, water management

Residents' ECPHM Session

O-RES-004

Effect of soy on faecal dry matter content and excretion of Brachyspira hyodysenteriae in pigs of different ages

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Introduction: *Brachyspira (B.) hyodysenteriae* is the causative agent of swine dysentery (SD), and can be detected by polymerase chain reaction (PCR) in porcine faeces. However, diagnostic sensitivity of testing faeces by PCR is low in case of subclinically infected pigs. Feeding high levels of soy increased clinical symptoms of SD in previous studies.
The aims of the present study were to investigate the effect of a high soy diet applied over two days on the general health condition of pigs, on the faecal dry matter content (FDMC) and on the faecal excretion of *B. hyodysenteriae* in faeces. An enhance excretion would improve the diagnosis in subclinically infected herds.
Materials and Methods: In total, 200 pigs of different ages from five farms with a recent diagnosis or presumptive diagnosis of *B. hyodysenteriae* infection were included in the study. Per farm, 40 pigs were randomly assigned to the control group (20 pigs; group C) or to the treatment group (20 pigs; group T). Pigs of group C received the farm's standard diet, whereas in the group T, half of the daily feed ration on day 0 (D0) and day 1 (D1) was replaced by pure soy. On D0, D2 and D6 a clinical examination with focus on general health condition, feed intake and rectal body temperature was performed in all pigs. Furthermore, rectal swabs and faecal samples of every pig were obtained and submitted to PCR for *B. hyodysenteriae* and FDMC analysis, respectively. A modified microwave method was used to determine the FDMC in triplets. The outcome was statistically analysed.
Results: All pigs in both groups consumed the diet. None of the pigs showed signs of reduced general health conditions or specific signs of SD throughout the study period. In grower pigs (56 pigs) the soy feeding resulted in a statistically significant decrease in the FDMC on D2 of 2.5 per cent compared to group C (58 pigs). In finishers and sows (39 pigs) the soy feeding resulted in a significant increase in the FDMC on D2 of 2.0 per cent compared to group C (40 pigs). The overall detection rate of *B. hyodysenteriae* by PCR at the three days of testing (597 samples) varied from 0.5 to 1.5 per cent and no statistically significant differences between group C and T were detected.
Conclusion: The present study showed that a high soy diet applied over two days significantly influenced the FDMC in growers, finishers and sows under field conditions, but did not improve the detection rate of *B. hyodysenteriae* using conventional PCR. Further investigations with more sensitive diagnostic methods are needed to prove a potential influence of a high soy diet on the detection rate of *B. hyodysenteriae* in subclinically infected herds.

Disclosure of Interest: None Declared

Keywords: Diagnostics, Enteric disease, Pigs

Residents' ECPHM Session

O-RES-005

Benchmarking finishing pig farms in the Netherlands using technical performance, antibody Elisa's and acute phase proteins

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Introduction: Although a lot of data is processed in the Netherlands regarding the production of finishing pigs, most of these data (like carcass traits, growth, feed conversion and infections) is kept isolated and not linked together. To gain more insight in the relation between technical data and the possible interactions with different infections a monitoring program was set up.
Materials and Methods: Farmers with finishing pigs in the Netherlands were given the opportunity to join the program. A protocol was provided for the attending vet to bleed 10 pigs within one week before slaughter for antibody serology using commercial available Elisa's (PRRS, PCV2, Mycoplasma, Influenza A, APP APX IV, *Lawsonia* and *Salmonella*) and to bleed 12 pigs 4-6 weeks before slaughter for the acute phase protein Pig-MAP. Blood samples were analyzed by the laboratory of the Dutch Animal Health service (GD Deventer). Technical data over the preceding period of 4 months of the farms were collected from the management systems (growth, FCR, mortality) and slaughterhouse (muscle thickness, backfat, carcass weight, pleuritis, pneumonia, discarded livers). Carcass weight was used to correct muscle thickness and backfat thickness. Data was benchmarked using a 1 to 5 scale (1 inadequate; 3 = average; 5 best in class). Used criteria were benchmarked either specific for the genetic lines (FCR, ADG, muscle and backfat thickness), the slaughterhouse (pneumonia, pleuritis & discarded livers) or the whole dataset (serology & Pig-MAP).
Results: Over 97 farms participated in the period July 2015-December 2015. Prevalence of the serology samples for the total number of blood samples (and farms): *Lawsonia* 76% (100%), PRRS 75% (88%), Influenza 64% (97%), PCV2 51% (87%), Mhyo 34% (52%). APP 55% (85%) *Salmonella* 22% (49%). PRRS-positive farms were significantly (p=0.05) associated with a higher risk for pneumonia (OR 6.4) and a lower growth (OR 3.67; p=0.04). PRRS had a tendency towards a higher FCR (OR 3.9; p= 0.07). The reference range for Pig-MAP for pigs in the finishing period was <1.20 mg/ml (= mean +2*SD). From the 50 herds positive for Mhyo, 35 vaccinated against Mhyo. Vaccinated herds had a tendency towards lower pleuritis at slaughter compared to farms without vaccination against Mhyo (10.7% vs 15.4%, p=0.16). Further results will be presented at the congress.
Conclusion: The combination of different data sources proved to be useful in monitoring finishing pig performance and benchmarking different farms can give more insights for improving the results on finishing farms. Respiratory pathogens like PRRS and Mhyo play an important role in finishing pig performance and are wide spread on farms in the Netherlands.

Disclosure of Interest: None Declared

Keywords: finishing pigs, Pig-MAP, technical performance

Residents' ECPHM Session

O-RES-006

Efficacy of early-life longtime Ceftiofur treatment in piglets on Streptococcus suis serotype 7 dynamics in a farm dealing with streptococcal diseases

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Introduction: In newborn piglets metaphylactic therapy with long-acting antibiotics, is a common approach to reduce losses due to streptococcal diseases on farms. The aim of this study was to assess the effect of early ceftiofur treatment on the isolation rates of *Streptococcus (S.) suis* and its impact on transmission.
Materials and Methods: In an Austrian farm with 120 sows and a history of disease caused by *S.suis* serotype 7 in the nursery, tonsillar and nasal swabs were taken from of 115 piglets of 19 litters immediately after birth before first contact with the environment. Within the first 10-14 living hours half of each litter (group A) got an intramuscular ceftiofur injection (Naxcel®, 100 mg/ml, Zoetis), the other half (group B) served as control group and was treated intramuscularly with 154 mM sodium chloride. On day 7 tonsillar swabs from 2 piglets (one treated, one untreated) out of each litter, and in week 14 from 4 pigs (two treated, two untreated) out of 8 litters were taken. Piglets were weaned with about 28 days and litters were mixed in the nursery. All samples were examined bacteriologically for *S. suis*. Isolates were characterized by multiplex PCR targeting housekeeping gene *gdh,* genes encoding capsule biosynthesis enzymes of serotypes 1, 2, 7 and 9 (cps1J, cps2J, cps7J and cps9H) as well as virulence-associated genes *epf*, *mrp*, *sly* and *arc*A.
Results: During the examination period no clinical signs occurred in pigs. 21 % of newborn piglets were positive for *S. suis* (15.2 % in nose, 11.3 % in tonsils). The genotypes of 28.5 % of *S. suis* isolates from nasal swabs and 30.4% from tonsillar swabs were identified as arcA+ gdh+ cps7J+ (serotype 7). Six days after treatment 39% of piglets in group A and 25% in group B were positive for *S.suis* on their tonsils. This difference was not significant. Treatment had no influence on the number of piglets becoming positive or negative during the first 7 days of life. On day 7 still one piglet (group A) was carrier of *S.suis* serotype 7 (arcA+, gdh+, cps7J+). At the age of 14 weeks, 100 % of the pigs were positive for *S. suis* in their tonsils and 44% were positive for serotype 7 (arcA+, gdh+, cps7J+). Daily weight gain from birth until weaning did not differ significantly between treated and not treated pigs.
Conclusion: In this study early-life treatment with long-acting ceftiofur had neither a preventive effect on *S.suis* colonization and transmission nor a positive effect onto daily weight gain. Colonization patterns were the same in both groups. Importantly, some piglets were colonized immediately after birth by the serotype 7 strain causing disease mainly after mixing of litters in weaning and growing piglets in this herd.

Disclosure of Interest: None Declared

Keywords: Cephalosporins, Streptococcus suis serotype 7

Oral Abstracts - Wednesday 08 June 2016

Residents' ECPHM Session

O-RES-007

Detection of respiratory pathogens in oral fluid; sampling recommendations in commercial conditions.

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Introduction: The aim of this study was to determine the frequency of respiratory pathogen detection in six farms with porcine respiratory disease complex (PRDC) by sampling oral fluids in 6 pens per farm at nine time points between weaning and finishing.
Materials and Methods:
Six wean-to-finish commercial farms were monitored for PRDC main primary agents in oral fluids (OF). Six pens per farm were repeatedly tested at 9 timepoints every 2 weeks from 5 to 21 weeks of age. One or several ropes were hung simultaneously in each pen (1 rope/25 pigs)). Nucleic acids in OF were extracted and analysed by quantitative PCR for PRRS, PCV2, SIV and *Mycoplasma hyopneumiae* (MagMax™ Pathogen RNA/DNA extraction kit and VetMAX™ qPCR, Thermo Fisher Scientific®). Clinical information and additional sampling material from sick and dead pigs were collected to corroborate findings in oral fluids. Correlation between CT values for duplicate ropes taken from the same pen were analysed by Pearson's correlation test.
Results: PRRSV was only detected in two farms; positive results were obtained from weaning age onwards. Pen-level prevalence ranged from 1/6 to 6/6 pens sampled and prevalence of detection was higher at the start of infection but tailed off over time.
PCV2 was detected in every farm at 5/9 to 9/9 timepoints. Viral loads $>10^6$ copies/mL OF were found in a farm with clinical PCV2 associated disease. Also high PCVD viral loads $>10^6$ copies/mL OF were found in farms in which the diagnostic process suggested a sub-clinic PCV2 case. Affected farms had levels over $>10^3$ copies/mL in several occasions.
Swine influenza virus was detected at 2-3 consecutive sample points in positive farms, even when infections were asymptomatic. At the peak of infection at least 5 out of 6 pens were positive.
M. hyo was detected in 4 out of 6 farms in consecutive sampling moments; the number of pens positive and Ct values were closely related to coughing and slaughter lesions.
Significant ($P<0.01$) correlations of the Ct values between pairs of ropes collected in the same pen were observed for PRRSV ($R^2=0.92$), PCV2 ($R^2=0.98$), SIV ($R^2=0.87$) and *M. hyo* ($R^2=0.92$).
Conclusion: Oral fluids are a successful platform to confirm PRDC causal agents in commercial conditions. In this study, repeatability of results at the pen level was strong and 2 week intervals gave a reliable herd level monitoring of the tested pathogens. Agreement of results between pens varied and this should be considered when designing sampling strategies. Consequently, surveillance and diagnostic testing needs careful planning to obtain reliable results; timing and number of pens to sample is very important.
Disclosure of Interest: J. Hernandez-Garcia Conflict with: Zoetis, Conflict with: University of Cambridge, N. Robben Conflict with: Thermo Fisher Scientific, D. Magnee Conflict with: Thermo Fisher Scientific, I. Dennis: None Declared, S. M. Kayes: None Declared, J. R. Thomson: None Declared, A. W. Tucker: None Declared

Keywords: Oral fluids, qPCR, respiratory disease

Residents' ECPHM Session

O-RES-008

Efficacy of several alternative measures to reduce antimicrobial usage in four European countries

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Introduction: The reduction of antimicrobial usage is especially a focus in modern pig production across countries. In order to move towards corrective actions, the objective of the study was to assess the effectiveness of potential alternatives to reduce antimicrobial usage at herd level.
Materials and Methods: In a prospective study, 67 pig herds with at least 100 sows and 500 fattening pigs located in Belgium, France, Germany and Sweden were recruited to implement intervention plans to reduce their antimicrobial usage. Alternative measures included the improvement of external or internal biosecurity (n=21 herds), vaccination (n=32), changes made to feed or waterquality or composite (e.g. zinc oxide) (n=42) and improved herd management (e.g. reduced density) (n=42). Herds were followed over one year and visited at least three times by the researcher together with the herd attending veterinarian. Annual antimicrobial expenditures were collected and compared to those of the year before intervention. Antimicrobial usage was measured as the treatment incidence (TI) and subsequently calculated by age category, antimicrobial class and administration route.
Results: Compliance with the initial intervention plans was high (86%), meaning that most of the farmers realized the implementation of predefined measures. Compared to the year before intervention, antimicrobial usage was significantly reduced following the implementation of alternative measures. In the median herd of the four countries, pigs were treated before intervention during 50 days from birth to their slaughter (i.e. 25% of an expected lifespan of 200 days), and after intervention during 32 days (i.e. 16%). Especially, the treatment incidence of suckling and weaning pigs was significantly reduced (by 37% and 54%, respectively). The usage of tetracyclines and polymyxines also significantly decreased. Although the reduction was not significant, the TI of critically important antimicrobials (i.e. fluoroquinolones and third-generation cephalosporins) was reduced. Treatment via feed and water, as well as parenteral treatment significantly reduced by 46% and 36%, respectively. Herds with a higher antimicrobial usage before intervention could achieve a bigger reduction.
Conclusion: Following tailor-made implementation of alternative measures, the reduction of antimicrobial usage in pig production is achievable. The reduction of the treatment incidence in the youngest age group (suckling and weaning pigs) and the reduction of group treatments via feed and water appear to be in line with the recent Guidelines on the prudent use of antimicrobials in veterinary medicine (European Commission, 2015).

Disclosure of Interest: None Declared

Keywords: alternatives, antimicrobial consumption, reduction

Residents' ECPHM Session

O-RES-009

Belly nosing by group-housed sows results in functional teat loss.

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Introduction: Belly nosing in pigs is described as a rhythmic rubbing of the belly by the nose of another pig. This behavior, originates from suckling behavior, and becomes non-functional when expressed by weaned piglets. Udder seeking or exploratory rooting behavior may explain belly nosing. When expressed by group-housed sows, the motivation of this behavior is unclear. This case reports the loss of functional teats due to mutilation by belly nosing and nipple suckling of group-housed sows during gestation.
Materials and Methods: The affected herd is a sow herd of 224 sows. Parity one to five are crossbred sows of Landrace and Yorkshire, whereas form parity 6 on sows are crossbred of Landrace and Large White. Gestating sows of all parities are housed together on concrete beds and slatted floors. Sows are fed by electronic feeding system (EFS). Since November 2014 sows were detected that missed functional teats at farrowing. Function loss was due to blind teats, missing nipples or necrosis of the nipples. In April 2015, 126 pregnant sows were examined for normal or abnormal teats and nipples.
Results: The average number of injured teats was 2.25 per sow. Thirty four sows were not affected and two sows missed as many as ten nipples. When examining the herd, one sow was found belly nosing and belly suckling. In addition, 104 sows were found to have lesions of the vulva, ranging from lacerations to absence of external genitalia in 49 sows. The aspect of the lacerations were suspected to be caused by the EFS. The abnormal wound healing and loss of vulva tissue may be related to the abnormal behavior of the sows. The intervention plan was based on detecting and culling of sows that showed belly nosing behavior, and repair of the EFS. In addition, straw bedding was administered. Since May 2015 six Landrace Large White crossbred sows (parity 6 and older), were detected belly nosing and culled. Since September 2015 no new cases were detected.
Conclusion: Based upon the visual observations and upon the result of the intervention - culling the sows with the belly nosing behavior - we conclude that this aberrant and harmful behavior caused loss of functional teats in this herd. No literature on belly nosing behavior in adult sows is available. How this behavior related to the observed vulva lesions, and what role genetics, feed or environmental enrichment plays, stays unclear. Excellent stockman-skills are essential to recognize and to successfully intervene in such a cases.

Disclosure of Interest: None Declared

Keywords: Belly nosing behavior

Oral Abstracts - Thursday 09 June 2016

S.SUIS

O-BBD1-001

A protein subunit vaccine provides substantial protection against virulent Streptococcus suis in pigs

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Introduction: *Streptococcus suis* is a bacterium commonly carried in the respiratory tract that is also one of the most important invasive pathogens of swine, commonly causing meningitis, arthritis, and septicemia. Due to the presence of many serotypes and high genotypic variability, efficacious vaccines are not readily available.
Materials and Methods: The selection of *S. suis* protein candidates for inclusion in a vaccine was accomplished by identifying fitness genes through a functional genomics screen, selecting predicted surface-associated proteins, and identifying proteins conserved across isolates to enhance the prospect of cross-protection. Five candidate proteins were selected for evaluation in a vaccine trial. Five-week-old, Caesarean-derived, colostrum-deprived pigs were distributed into groups as follows: group 1 pigs were vaccinated intramuscularly (IM) with Carbopol/AddaVax as adjuvant and intranasally with polyethyleneimine as adjuvant; group 2 pigs were vaccinated similarly but the IM adjuvant was EmulsigenD; groups 3, 4, and 5 were control groups given PBS mixed with the same adjuvants as groups 1 and 2 or no adjuvant, respectively. Pigs were given 2 doses of the vaccine 2 weeks apart and challenged with virulent *S. suis* by the intranasal route 2 weeks later to evaluate clinical protection.
Results: Two weeks following priming and boost serum IgG to each individual *S. suis* proteins was increased in vaccinated pigs and adjuvant formulation had a significant impact on responses, with higher titers for pigs in group 2 compared to group 1. Cytokines produced by peripheral blood mononuclear cells following restimulation with the protein pool were also highest in pigs from group 2. Subunit vaccination induced IgG reactive against *S. suis* bacteria from a number of different serotypes. Nine of 10 pigs in control groups 3-5 developed systemic *S. suis* infection and had to be euthanized. *S. suis* was cultured from systemic sites and lesions consistent with infection were present. In the 2 vaccinated groups, 3/6 pigs in group 1 and only 1/6 pigs in group 2 developed systemic disease and had to be euthanized.
Conclusion: Subunit vaccination with the *S. suis* proteins provided substantial protection from challenge with virulent *S. suis*. Both the magnitude of the immune response and degree of protection was dependent on the parenteral adjuvant given, suggesting this was the important delivery method for protection; however, a role for mucosal immunization in protection or priming of the immune response cannot be ruled out. The reactivity of the sera from vaccinated pigs against several diverse *S. suis* strains may indicate a potential for cross-protection that will need to be confirmed through additional studies.

Disclosure of Interest: None Declared

Keywords: protein subunit, Streptococcus suis, vaccination

Bacteriology and Bacterial Diseases
S.SUIS

O-BBD1-005

Novel molecular-markers to discriminate between systemic and non–disease associated Streptococcus suis isolates

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Introduction: *Streptococcus suis* is an important swine pathogen isolated in almost all countries with an established pig industry. Infection causes septicaemia, meningitis and arthritis - also contributing to pneumonia. Development of practical diagnostic and epidemiological tools to identify strains with potential to cause disease underpins effective prevention programs.
Materials and Methods: We performed whole-genome sequencing of 115 *S. suis* isolates sourced from UK farms in 2010. For each isolate well-defined clinical metadata was available, enabling the categorisation of isolates as being associated with systemic streptococcal disease or as being non-disease associated. A genome-wide association study (GWAS) was used to identify molecular markers consistently associated with isolates responsible for systemic disease. Three candidate markers were selected for evaluation in a proof of concept trial using a multiplex-PCR (mPCR) against the original isolate collection. Banding patterns were converted to test scores using a generalised linear model and then turned into a binary class decision by choosing a test cut-off identified using a receiver operating characteristic (ROC) curve. Classifier performance was compared against other tests currently used to predict 'virulence' such as belonging to serotypes 1-10 and 14 (as determined *in silico*); presence of the three genes *ef, mrp* and *sly*; and minimum core genome (MCG) classification. A further evaluation was done using an additional UK set of clinically phenotyped systemic (n=23) and non-disease associated isolates (n=50) from 2013.
Results: The classifier showed a sensitivity (Se) of 0.9 and specificity (Sp) of 0.79 when compared to the training set clinical metadata. Comparison against serovar as a predictor of virulence (serovars 1-10 and 14; Se: 0.98, Sp: 0.58) or prevalence of all three 'virulence-associated' genes *ef, mrp* and *sly* (Se: 0.77, SP: 0.97), or grouping by MCG scheme (Se: 0.77, Sp: 0.97) showed significantly superior performance for the new test (p.value<0.05; using DeLong's test for two correlated ROC curves). Test performance using the additional collection of 2013 isolates showed a Se of 0.87 and Sp of 1.0.
Conclusion: We used a GWAS to identify three molecular markers capable of discriminating systemic from non-disease associated isolates of *S. suis*. The mPCR performed significantly better than currently available 'virulence'-associated tests. Further work will attempt to identify additional molecular markers associated with specific pathology e.g. meningitis or pneumonia, and will incorporate global isolates of *S. suis*.

Disclosure of Interest: None Declared

Keywords: Diagnostic, Streptococcus suis, Virulence

S.SUIS

O-BBD1-003

An improved method of Streptococcus suis serotyping and vaccine efficacy

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Introduction: *Streptococcus suis* is a gram positive bacterium that causes arthritis and fatal meningitis in young pigs. Currently, 35 different serotypes have been identified and serotype 2 is most commonly associated with the disease in the U.S. Serotyping results from isolates sent to diagnostic laboratories for testing are often non-typeable or ambiguous using current methods. In the present study, we used next generation sequencing to identify the serotype of several isolates. This method appears to be robust and could correctly assign a serotype to nearly all isolates tested. We recently completed a vaccination-challenge study demonstrating that the *S. suis* capsule is the primary target of protective immune responses.
Materials and Methods: The genomic DNA from *S. suis* isolates was extracted, libraries constructed, and sequenced on a MiSeq instrument. Reads were mapped to reference genomes to identify the serotype of each isolate. The results were compared against those obtained from traditional testing. To demonstrate the importance of the capsule in vaccine formulation, ten naïve pigs were vaccinated with one of three different preparations: A) A capsular conjugate vaccine; B) An inactivated whole cell vaccine; C) Mock vaccine. Animals were challenged two weeks after their last vaccination with the same isolate used for vaccine preparation. Clinical symptoms and mortality were evaluated post-challenge.
Results: The serotyping method described here can be used to rapidly establish a serotype for *S. suis* isolates and more is reliable than standard serotyping methods such as PCR and serum agglutination. In addition, this method allows a more holistic examination of differences present in the capsular locus of strains from different serotypes. Our data indicate that field isolates of *S. suis* have a complex and plastic genome. The capsular locus is amenable to extensive recombination. Thus, capsular loci possess a complex mosaic structure. Our vaccination-challenge study demonstrates the importance of the capsule in inducing a protective immune response based on mortality scoring. When pigs were given a lethal challenge, a vaccine manufactured using capsular components was able to protect a majority of the pigs. When a whole cell vaccine was used, nearly complete protection was achieved, demonstrating the utility of whole cell vaccines for protection of animals when serotypes are well matched.
Conclusion: In this study, when used in conjunction with a robust serotyping method, inactivated vaccines provided protection against a lethal *S. suis* challenge.

Disclosure of Interest: None Declared
Keywords: None

S.SUIS

O-BBD1-004

Use of MALDI-TOF MS for identification of Streptococcus spp. from swine clinical samples

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Introduction: The genus Streptococcus is composed of spherical morphology bacteria that grow in variable-length strings. Are cocci Gram-positive, catalase negative, facultative anaerobes and not mobile. The species most described in swine industry is *Streptococcus suis*, but several species can infect swine and cause different clinical signs. Traditional microbiological methods were used to identify the *Streptococcus* genus; however, the species present broad phenotypic variation, making it difficult for their identification or even differentiation just by these methods. This study intended to evaluate the use of MALDI-TOF MS technique for the identification of *Streptococcus* different species capable of causing disease in swine.
Materials and Methods: A total of 250 strains were studied with *Streptococcus* morphologic characteristics isolated from pigs presenting clinical signs of encephalitis, arthritis, pneumonia, metritis, infection urinary or septicemic, from different Brazilians states, between 2001 and 2014.
The Streptococcus-like colonies were reactivated to perform the ribosomal protein extraction required for the protein profiles differentiation. The strains were screened by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) identification. Mass spectra were acquired using a Microflex™ mass spectrometer (Bruker Daltonik) and identified with manufacturer's software MALDI BioTyper™ 3.0. For the species confirmation, 16S rRNA gene sequencing was performed. A phylogenetic tree was constructed using the maximum-likelihood method.
Results: The strains were grouped according to the isolation sites, 46.8% were isolated from de central nervous system (117/250), 32.8% from respiratory tract (82/250), 5.2% from reproductive and urinary tract (13/250), 10% from articulation (25/250), 5.2% from other sites (13/250). Among studied strains, 86% (215/250) were identified as *Streptococcus suis* with score > 2.00 and 14% (35/250) were identified as different species of the same genus, including *S. hyovaginalis, S. oralis, S. hyointestinalis, S. sanguinis, S. henryi, S. alactolyticus, S. plurianimalium, S. dysgalactiae, S. gallinaceus, S. gordonii, S. gallolyticus* e *S. mitis.* The results obtained with MALDI-TOF MS technique were concordant withidentification by16S rRNA gene sequencing.
Conclusion: Given the data analysis, it can be concluded that the MALDI-TOF MS technique allows the identification of other *Streptococcus* species capable of causing disease in swine that are not often identified in routine diagnostics.

Disclosure of Interest: None Declared

Keywords: Identification, MALDI-TOF, Streptococcus

Oral Abstracts - Thursday 09 June 2016

LAWSONIA

O-BBD1-002

INVOLVEMENT OF MICE (Mus musculus) IN THE EPIDEMIOLOGY OF PORCINE PROLIFERATIVE ENTEROPATHY

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Introduction: Proliferative enteropathy is an enteric infectious disease caused by *Lawsonia intracellularis.* Interspecies transmission and the role of rodents in the epidemiology of disease are not fully understood. *L. intracellularis* DNA was detected in rodent feces trapped in hog farms known to be positive for bacteria. The aim of this study was to evaluate the fecal-oral transmission of *L. intracellularis* between mice and pigs.
Materials and Methods: The study was divided in two parts. The first part aimed to check if mice could be infected by feces from *L. intracellularis* experimentally infected pigs. Thirty-four Swiss mice were allocated in 4 boxes, and received 20g/box of feces of experimentally infected pigs PCR positive for *L. intracellularis* (M1), for four consecutive days. Twelve other mice received swine negative feces (M2). Pool of mice feces were collected from each box in alternating days post exposition (dpe). All mice were euthanized on day 28 dpe and intestinal samples were collected for immunohistochemistry (IHC) for *L. intracellularis.* The second part aimed to test if pigs could be infected when exposed to PCR positive feces of *L. intracellularis* experimentally infected mice. Twelve 5-week-old pigs received feed mixed with PCR positive mice feces (S1), while other two pigs received negative mice feces (S2), for four consecutive days. Pig feces and serum samples were individually collected on 0, 7, 14, 21, 28 dpe. All pigs were euthanized on day 30 dpe and intestinal samples were collected for IHC. Serum samples were tested by IPMA for IgG anti-*L. intracellularis.* Pig and mice feces were tested by qPCR.
Results: All pigs and mice used in the present study were negative for *L. intracellularis* at the beginning of the studies. Animals in M2 and S2 remained negative until the end of the experiments. In the first study, the amount of *L. intracellularis* provided to M1 boxes per day was between 10^6 and 10^8. Mice eliminated on average 10^4 bacterial in all collecting days. Three mice from the exposed group were positive by IHC at the end of the study. In second study, the animals in S1 received on average 10^5 bacterial per day and 10 were infected by *L. intracellularis,* based on qPCR and/or immunohistochemistry positive results. The pigs eliminate average 10^4 *L. intracellularis*/g of feces, detected at 14 to 30 dpe. Nine pigs seroconvert between 21 and 30 dpe. Using IHC, 50% of the pigs were positive.
Conclusion: *L. intracellularis* experimentally infected mice and pigs can infected each other, therefore, rodent have to be considered as player in the epidemiology of the disease in hog farms.
Acknowledgements: FAPEMIG for financial support.
Disclosure of Interest: None Declared
Keywords: Epidemiology, PPE, qPCR

E.COLI

O-BBD2-009

Can pooled pen floor samples be used for diagnosing Escherichia coli F18 positive diarrhoeic nursery pigs?

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Introduction: Bacterial culture and PCR testing for toxin genes from pig faecal samplings has been used as a diagnostic tool for diagnosing ETEC in faecal samples from individual diarrhoeic nursery pigs. qPCR analysis on “sock samples” (pooled faecal samples collected on socks) has in the recent years become more popular due to the ability to analyse samples from groups of pigs. The purpose of this study was to investigate if testing of pooled faecal pen floor samples (PFP) can be used for diagnosing ETEC in diarrhoeic nursery pigs. Sensitivity and specificity of two diagnostic methods; bacterial culture and qPCR analysis of PFP were evaluated. Results from bacteriological testing of faecal samples in individual diarrheic pigs were used as golden standard.
Materials and Methods: Pigs from three commercial nursery facilities in the eastern part of Denmark with a diarrhoea prevalence >25% in pens two to four week after weaning was investigated. Faecal samples from 86 diarrhoeic pigs in 31 pens and a pen floor sample were collected and cultured on blood agar. After culture *E. coli* isolates were analysed by PCR for the adhesion factor F18 and the toxin genes; (VT2e, STa, STb and LT). If one isolate from one pig faecal samples in the pen possessed gens fore F18 fimbriae and one or more toxins the pen was classified as virulent *E. coli* positive (gold standard). The PFP samples were cultured and classified as virulent *E. coli* positive if one isolate from the sample possessed genes for F18 fimbriae and one or more toxins. Furthermore, the PFP samples were qPCR analysed for F18 gens with a cut-off value of $1.5x10^3$ bacteria/g faeces. The association between virulent *E. coli* positive PFP by bacterial culture and occurrence of F18 positive *E. coli* diarrhoeic pigs and the association between F18 positive *E. coli* by qPCR analysis and occurrence of F18 positive *E. coli* diarrhoeic pigs were tested by chi^2 test and sensitivity and specificity was calculated.
Results: There was a strong association between detection of virulent *E. coli* strains from PFP samples by culture and occurrence of virulent *E. coli* positive diarrhoeic pigs (p-value=0.001, SE=90.9, SP=81.3). Furthermore, there was a significant association between detection of F18 gens by qPCR in PFP and occurrence of F18 positive *E. coli* in diarrhoeic pigs (p-value=0.015, SE=83.3, SP=68.4).
Conclusion: The results of the study showed that both culture and qPCR testing of pen floor samples can be used as a diagnostic method for detection of groups of diarrhoeic nursery pigs positive for virulent F18+ *E. coli.* The highest sensitivity was observed when culture was used rather than qPCR.
Disclosure of Interest: None Declared
Keywords: diarrhoea, E. coli , nursery pigs

E.COLI

O-BBD2-007

Comparison of histology and PCR to diagnose Edema Disease

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Introduction: Edema Disease (ED) is one of the main causes of mortality in weaned pigs. It is caused by shigatoxin producing *Escherichia (E.) coli*. After the preliminary diagnosis based on clinical signs is made, the disease can be confirmed by taking intestinal samples from dead piglets. Commonly, these samples are first submitted to microbiological culture specific for *E. coli*. Secondly, the colonies of *E. coli* are further identified by their genes encoding for toxins and adhesion factors via multiplex PCR (mPCR). Sometimes these results can be false negative if the pigs have been dead too long or have been previously treated with antimicrobials. A single PCR for the *Stx2e* gene directly from feces (without prior incubation of samples in culture media) may solve this problem.

Materials and Methods: Twenty-one pigs from 14 farms were selected from those animals sent in for routine analysis to a diagnostic laboratory. After the preliminary diagnosis of ED made by the individual herd veterinarian had been substantiated by gross pathology, the following samples were taken from each pig: one swab from the small intestine for the common procedure of microbiology and subsequent mPCR, one naïve fecal sample from the rectum for the new single PCR, one sample each of the brain, spinal cord and intestine for histology. Lesions consistent with ED in the histology were considered the gold standard. A pig was considered ED positive if the gene was found in the PCR. The same classification applied to culture and mPCR.

Results: PCR results obtained from samples of 18 pigs were in accordance with histological results with 12 pigs being positive and six pigs being negative for ED. Discordant results were obtained for two pigs which were negative in histology but positive with PCR and also in microbiology plus mPCR. In one case both histology and PCR were positive, whereas the microbiology was negative.

Conclusion: First results indicate that the single PCR directly from feces may be an alternative method to confirm the diagnosis of ED if it is not possible to take samples for microbiology and subsequent mPCR and/or histology. However, this was only a preliminary study with a small sample size. Further studies are needed comprising a larger number of pigs not only suffering from ED but also healthy pigs to assess the sensitivity and specificity of the PCR method as compared to histology.

Disclosure of Interest: V. Gotter Conflict with: IDT Biologika, S. Hillen Conflict with: IDT Biologika, G. Reiner: None Declared, H. Willems: None Declared

Keywords: Diagnostic, Edema Disease

BRACHYSPIRA

O-BBD2-006

In vitro organ culture (IVOC) of porcine colon response to infection by "Brachyspira hampsonii"

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Introduction: Mucohaemorrhagic diarrhea in pigs has global distribution and a remarkable economic impact due to reduced performance of animals, increased mortality and medication costs. A critical knowledge gap exists regarding the disease pathogenesis. Multiple animal models have been used to demonstrate *"B. hampsonii"* pathogenicity to pigs. Animal models limit pathogenesis studies since the onset of the host cellular response and tissue damage stages are not clinically detected for sampling. In this study, *in vitro* culture of porcine colon was used to study the early interaction between "*B. hampsonii"* and its porcine host. *In vitro* organ culture (IVOC) involves the maintenance of tissue explants derived from healthy subjects *ex vivo* for several days, enabling accurate control of host exposure to pathogen and timed sampling after inoculation.

Materials and Methods: IVOCs from porcine colon were exposed to *"B. hampsonii"* for 12 hours. Explants (n=400) from ten, 8-week old pigs were prepared and randomly assigned to inoculated and control groups. Inoculated explants were exposed to a 48-hour-old pure culture of *"B. hampsonii",* while controls received sterile culture broth. Explants were fixed in 10% buffered formalin (n=2/pig/time) or RNAlater (n=2/pig/time) at 0, 2, 4, 8 and 12 hours post infection (hpi). Samples were analysed by optical microscopy (H&E and Warthin-Faulkner stains) and quantitative PCR (GAPDH, e-cadherin, IL-1α, IL-8, INF-γ and TNF-α mRNA levels).

Results: Significant differences in the number of dead cells within crypts were observed between groups when all time points were taken in account, and at 2, 4 and 8 hpi ($P \leq 0.001$, GEE). A trend was observed at 12 hours ($P=0.07$, GEE). Catarrhal exudate containing variable amounts of mucus, necrotic epithelial cells and bacteria increased in thickness over time, with significant differences observed between groups when all time points were considered together ($P<0.01$, GEE), and after 4 and 8 hpi ($P<0.05$, GEE). A trend was observed at 12 hours ($P=0.06$, GEE). Spirochaetes were observed in the mucus layer and in contact with necrotic, exfoliated cells within 2 hours, and after 4 hours within crypts and the *lamina propria*. No statistically significant differences were observed in mRNA levels between inoculated and control explants for any of the targeted genes during the studied period.

Conclusion: Host response to "*B. hampsonii"* within the first 12 hours of exposure was characterized by progressive increases in the apical catarrhal exudate thickness and the number of dead cells within crypts. These findings are similar to histopathological findings described after *in vivo* infection of pigs with "*B. hampsonii".*

Disclosure of Interest: None Declared

Keywords: Brachyspira hampsonii, IVOC, Pathogenesis

Oral Abstracts - Thursday 09 June 2016

BRACHYSPIRA

O-BBD2-008

Virulence-associated genes in Brachyspira hyodysenteriae isolates from German pig herds with different health statuses

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Introduction: Infection with *Brachyspira hyodysenteriae* can lead to swine dysentery (SD), although sometimes isolates of the spirochaete have been recovered from apparently healthy herds. It has been suggested that strains that lack some plasmid genes may have reduced capacity to colonise and cause disease. The aim of this study was to characterise *B. hyodysenteriae* isolates from German herds with and without disease to determine their diversity and whether their plasmid gene content differed.

Materials and Methods: Thirty-five *B. hyodysenteriae* isolates were recovered from 24 German herds. Ten herds had a history of SD, three had mild diarrhoea of unknown aetiology, eight were of uncertain disease status, and three had no disease. Multiple isolates (n= 3, 4 and 6) were obtained from the latter three herds.

The isolates were subjected to multilocus sequence typing (MLST) and PCRs targeting the six plasmid-encoded genes that have been described as putative virulence genes in *B. hyodysenteriae*. Isolates with all six genes present were recorded as predicted “virulent”, those with all six genes missing were scored as “avirulent”, those with five genes missing were recorded as “low virulence”, and those with two genes missing were scored as “reduced virulence”.

Results: Multiple isolates from the same three herds without disease had the same sequence type (ST), but each herd had a different ST. Overall seven new STs and six previously described STs were identified amongst the 35 isolates. Three of the new STs were from the herds without disease. The previous STs corresponded to strains from Germany (ST52, ST112, ST114, ST118 and ST120), Belgium (ST52), Italy (ST52) and the USA (ST104).

Four isolates had all six plasmid genes present, and these were all from farms with SD. Isolates from the other six farms with SD were variously “avirulent” (n=3), “low virulence” (n=2) or “reduced virulence” (n=1). Potentially other virulent isolates may have been present on these farms, but were not isolated and tested.

Four herds had a history of diarrhoea, and the isolates were “low virulence” (n=2), “reduced virulence” (n=1) or “avirulent” (n=1). The eight herds with uncertain disease status all had isolates that were either “avirulent” (n=4), “low virulence” (n=3) or “reduced virulence” (n=1). For the multiple isolates from the three herds without disease, the strains had the same virulence gene profiles, which were “reduced virulence” in one herd and “low virulence” in the other two.

Conclusion: The study supports the suggestion that *B. hyodysenteriae* isolates from healthy herds are likely to lack some or all of the six plasmid-encoded genes that have been linked to virulence.

Disclosure of Interest: None Declared
Keywords: Brachyspira hyodysenteriae, MLST, Virulence

CLOSTRIDIA

O-BBD3-010

The Use Of Probiotics As An Aid In The Control Of Clostridium Difficile Associate Disease In Neonatal Pigs

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Introduction: *Clostridium difficile* (CD) is one of the most important cause of enteric disease in neonatal piglets. *C. difficile* infection (CDI) is often associated with disequilibrium of the intestinal microbiota. Reestablishment of the intestinal microflora through the use of probiotics is the therapy adopted in human medicine since vaccines are not available. The project objective was to evaluate the use of *Lactobacillus* sp. and a non-toxigenic *C. difficile* strain (NTCD) as probiotic alternatives to prevent the development of CDI in piglets.

Materials and Methods: Two probiotic types were utilized: 1) NTCD 2) *Lactobacillus* sp. commonly present in commercial yogurt products. 150 caesarian derived piglets were divided in 6 treatment groups. Piglets were individually housed in a BSL2 facility. Treatment groups are summarized as follows: GROUP 1: negative control (n=10), GROUP 2: $2x10^6$ heat-shocked NTCD spores (n=13), GROUP 3: *Lactobacillus* (n=14), GROUP 4: positive control (n=35), GROUP 5: NTCD plus $2x10^6$ spores of toxigenic strain of *C. difficile* (n=34), and GROUP 6: *Lactobacillus* plus toxigenic *C. difficile* (n=44). Probiotic treatment was intragastrically administrated within the first two hours-of-life according to experimental design; 16 hours later pigs were intragastrically challenged with the toxigenic strain of *C. difficile*. Pigs were monitored for 72 hours post inoculation and then necropsied. Gross and microscopic lesions were individually scored. ELISA toxin assay was performed on fecal content.

Results: Pigs in GROUP 5 presented with significantly lower mesocolonic edema scores when compared to pigs in GROUPS 4 and 6 ($P = 0.01$). All animals were *C. difficile* toxin ELISA negative at the beginning of the experiment. At necropsy, ELISA results showed that pigs in GROUP 5 had lower levels of toxin when compared to pigs in GROUPS 3, 4 and 6;however, these results were not significantly different ($P = 0.12$). Pigs in GROUP 4 had higher histologic scores when compared to pigs in other groups and pigs in GROUP 5 presented lower microscopic scores when compared to pigs in GROUPS 3, 4 and 6. Histologic scores in GROUP 5 were similar to those for piglets in GROUPS 1 and 2. Presence of mesocolonic edema was correlated with histologic scores and ELISA results ($P < 0.001$) with respective Spearman coefficients of 0.4064 and 0.3442.

Conclusion: This study demonstrated that the administration of NTCD decreased prevalence of toxin-positive piglets, reduced mesocolonic edema and microscopic lesions, suggesting a benefit to administration of NTCD as a competitive exclusion technique to prevent CDI in piglets.

Disclosure of Interest: None Declared
Keywords: Clostridium difficile, Porcine, Probiotic

ACTINOBACILLUS

O-BBD3-013

Role of T cells in the immune-pathogenesis of porcine contagious pleuropneumonia

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Introduction: Porcine contagious pleuropneumonia caused by *Actinobacillus pleuropneumoniae* (APP) remains one of the major causes of poor growth performance in pig herds. After recovering from the acute phase, pigs often become carriers harbouring the pathogen in tonsils, nares and chronic lung lesions. While most of the literature focuses on the role of the humoral response in attenuating the occurrence of acute symptoms, the porcine T-cell response to APP has been poorly characterised so far. Therefore, the presented study aimed at providing an overview of the host immune response during persistent infection with particular emphasis on cytokine production by APP-specific T cells.
Materials and Methods: Twenty pigs were intranasally inoculated with 2 x 10^4 CFU/ml of APP serotype 2, by means of an atomization mucosal device to mimic the natural infection. Ten pigs were humanely euthanized at the acute phase (6-10 dpi) and the remaining ten at the chronic phase of APP infection (27-31dpi). Nasal, tonsillar and blood samples were collected weekly. Salivary glands, tonsils, BALF, lung tissues and tracheobronchial LNN were harvested at necropsy. Peripheral blood mononuclear cells (PBMC) and lymphocytes isolated from tonsils, lung samples and tracheobronchial LNN were phenotyped by determining the expression of CD4, CD8α and TCR-γδ. Furthermore, their ability to produce IL-17A, IL-10 and TNF-α was analysed. For this purpose, cells were stimulated overnight with a crude capsular extract of APP serotype 2.
Results: Clinical records, microbiological investigations and pathological findings confirmed the induction of a chronic APP infection. First results point to the induction of APP-specific IL-17A producing T cells both in acute and persistent infection as well as IL-10 producing T cells in persistent infection. This may indicate that IL-10 production plays a role in the persistence of APP, but further investigations are needed to confirm this hypothesis.
Conclusion: In conclusion, this study provides first hints that T cells are involved in the immune-pathogenesis of porcine contagious pleuropneumonia.
Disclosure of Interest: None Declared
Keywords: APP, persistence , T cells

ACTINOBACILLUS

O-BBD3-012

Spreading of Actinobacillus pleuropneumoniae to different body tissues of the pig during acute phase of infection

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Introduction: *Actinobacillus pleuropneumoniae* (*A. pleuropneumoniae*) is one of the most important pathogens in pork production. In contrast to *H. parasuis*, which can also cause severe polyarthritis, polyserositis and meningitis, *A. pleuropneumoniae* is described as a lung pathogen leading to porcine pleuropneumonia. Nevertheless there are several case reports of arthritis, osteomyelitis, hepatitis or meningitis where *A. pleuropneumoniae* was the only detectable pathogen. Thus the aim of this study was do investigate the spreading of *A. pleuropneumoniae* to different body tissues during the acute phase of experimental aerosol infection.
Materials and Methods: A variety of samples from 10 pigs (3 slightly (G1), 3 moderate (G2) and 4 severely diseased (G3)), experimentally infected with *A. pleuropneumoniae* serotype 7, were cultured for *A. pleuropneumoniae*. Swabs from the pleura, pericardium, peritoneum, meninges, carpal and tarsal joint as well as tissue samples from liver, spleen and kidneys were plated on selective medium for *A. pleuropneumoniae* at day 7 p.inf. or earlier if euthanasia was necessary. The amount of pleural and abdominal fluid was measured, samples with and without EDTA were taken and the number of colony forming units (cfu)/ml was determined. From two animals blood samples after per-acute death were analysed.
Results: Both blood samples were highly positive for *A. pleuropneumoniae*. In group G1 one animal was tested positive within the peritoneal swab sample, in all other samples *A. pleuropneumoniae* was not detected. In group G2 *A. pleuropneumoniae* could be cultured from the pleural, pericardial, peritoneal, liver, kidney samples of all three animals. Two animals were also positive in the meningeal and tarsal swabs and one was positive in the carpal swab. In Group G3 all animals were positive in the pleural, pericardial, peritoneal, liver, spleen, and kidney samples. One was positive in the meningeal probes and none of them was positive in the carpal and tarsal joints. All of the moderate and severely diseased animals had an increased amount of pleural fluid and 6 of 7 animals had an increased amount of abdominal fluid. The cfu differed between 1.0 x 10^3 and 3.1x10^8 /ml. The detection efficiency of A. *pleuropneumoniae* by bacteriological culture was significantly reduced in the EDTA samples ($p < 0.001$).
Conclusion: Apparently *A. pleuropneumoniae* spreads regularly to the whole body during the acute phase of infection most likely by bacteraemia. However there were no histological alterations seen in the sampled tissues. Further investigations are needed to identify factors that lead to the development of alterations in these organs.
Disclosure of Interest: None Declared
Keywords: Actinobacillus pleuropneumoniae, arthritis, spreading

Oral Abstracts - Thursday 09 June 2016

ACTINOBACILLUS

O-BBD3-011

Evaluation of maternally-derived antibodies against Actinobacillus pleuropneumoniae (APP) in piglets from APP-positive herds in Belgium

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Introduction: The aim of this study was to investigate maternally-derived antibodies against *Actinobacillus pleuropneumoniae* (APP) in piglets from APP-positive pig herds. Data originates from APP Check, a service to evaluate maternal immunity based on antibodies against APP toxins ApxI, ApxII, ApxIII and Outer Membrane Protein (OMP) of APP, with the aim to optimize the strategy for piglet vaccination.

Materials and Methods: For each herd, a cross-serological investigation was performed on 5 age groups: 6, 8, 10, 12 and 14 weeks, with 5 samples per group (n = 15 farms). Four farms also submitted blood samples from 4 week old piglets. All samples were tested for antibodies against ApxI, ApxII and ApxIII and OMP by an indirect-ELISA test (In-house-test, MSD AH Service Lab, Boxmeer, The Netherlands). Cut off value for clinical protection is predicted at 9 Log 2. Antibodies against ApxIV were analyzed by IDEXX ApxIV ELISA®. Blood samples were taken prior to implementation of APP vaccination in piglets. Only herds without APP vaccination in sows were included in the analysis.

Results: Mean antibody levels (presented in Log 2 scale ± SD) against ApxI were 10.9 ± 1.9, 9.7 ± 1.7, 8.9 ± 1.6, 8.3 ± 1.6, 7.5 ± 1.1 and 8.6 ± 1.9 for 4, 6, 8, 10, 12 and 14 weeks of age. Similar antibody levels were found against ApxIII and OMP. Antibody levels against ApxII showed a similar trend but at a significantly higher level at 6, 8, 10 and 12 weeks ($p<0.05$). The average levels of maternal antibodies against ApxI, ApxIII and OMP dropped below 9 Log 2 between 6 and 8 weeks and reached a minimum at 10 weeks. The ApxIV results did not show a similar trend because in 46% of the farms, all samples remained positive up to and including 10 weeks. The number of ApxIV-positive samples decreased after 10 weeks. Exposure to APP and an active immune response was indicated at five farms by an increase of antibody levels against one or more Apx toxins and OMP between 10 and 14 weeks.

Conclusion: Duration of maternal immunity against APP is on average 6 to 8 weeks based on ApxI, ApxIII and OMP serology. The relatively higher levels of ApxII antibodies might be explained by subsequent infections with different ApxII-producing APP bacteria or by cross-reaction with antibodies against similar RTX toxins from other bacteria. With the qualitative outcome of the ApxIV ELISA test (positive/negative) we were not able to detect a regression of maternal immunity in piglets up to and including 10 weeks. Therefore, quantitative serological tests are preferred for monitoring of maternal immunity against APP and determination of a “tailor-made” vaccination strategy.

Disclosure of Interest: None Declared
Keywords: Actinobacillus pleuropneumoniae, Maternal immunity

O-WN1-001

Relationships between weight, head morphology-assessed IUGR status and survival in commercial piglet production

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Introduction: Production improvements in the global swine industry have been partly produced by increasing selection for hyperprolific sows, resulting in larger litter sizes while also increasing the variation in piglet birthweight. This has increased the number of small and/or undersized piglets, which have been exposed to differing degrees of intrauterine growth restriction (IUGR). Most IUGR piglets are classified as being of low birth weight, however, there is large variation in weight of both non-IUGR and IUGR piglets. The aim of this study was to look at the relationship between weight, IUGR status assessed from head morphology and survival.
Materials and Methods: All piglet births (n=8507) were recorded for a 20 week period in a population containing 650 Landrace sows crossed with either White Duroc sires (WD; 6) or Large White sires (LW; 13), with data gathered at first processing (18-24 hours after birth) on piglet weight, gender, head shape, and date and reason for any death. All piglets remained in their birth litters until processing, but were fostered thereafter. All gilts were tagged with individual identification but boars were left untagged and were unidentifiable after processing. The degree of intrauterine growth restriction (IUGR) was assessed visually from head morphology and piglets were classified as normal (1), slight IUGR (2) or IUGR (3; steep, dolphin-like forehead and wrinkles perpendicular to the mouth).
Results: Significant effects (GLM model) on weight were head shape (P<0.001) and sire breed (P<0.001). Piglet sex was not significant although the interaction between head shape and sex was significant (P=0.003). Head shape 1 piglets were heaviest (fitted means ±SE; n=7184, 1562g±3.7), then head shape 2 (n=1108; 982g±3.0), and head shape 3 were lightest (n=215; 677g±21.3). The proportion of piglets dead at birth (0.05, 0.10, 0.14), dead between birth and processing (0.03, 0.07, 0.15), and between processing and weaning (0.04, 0.05, 0.20) were all significantly (P<0.001) associated with head morphology score (head shape 1,2,3 respectively). Binary logistic regression showed significant influences of weight (P<0.001), breed (P<0.001), sex (P<0.001) and head morphology score (P=0.039) on the probability of surviving to processing.
Conclusion: In conclusion, head morphology explains variance in piglet survival to weaning in addition to birth weight and the genetic contribution to these indicators is being assessed. The interactive effects of birth weight and head morphology on piglet survival require more detailed anatomical and physiological investigation. This research was funded by the EU FP7 Prohealth project (no. 613574).

Disclosure of Interest: None Declared

Keywords: breed, IUGR, Piglet survival

O-WN1-002

Improvement of animal welfare and productivity in pigs by using social genetic effects

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Introduction: The demand for higher efficiency in a world with an increasing competition between food for humans and feed for animals. This has resulted in 1% to 3% genetic progress per generation in commercial breeding programs. In developed countries there is also more attention on different aspects of animal welfare of which behaviour is one of the most important focus point. In pigs, mal-behaviours such as aggression and tail biting are the main behaviours under investigation.
Materials and Methods: A very promising selection method with the potential to improve both animal welfare and economic output is the use of social genetic effects (SGEs) in breeding programs. A social genetic effect is a heritable effect of one individual on the trait value of another individual. The breeding approach using SGEs incorporates both the direct genetic effect due an individual, and the genetic effect an animal has on its pen mates into the trait value of the individual. While traditional methods focused on individual performance only, this strategy could improve growth in pigs, as well as the behaviour of pigs which are housed in groups. A one generation selection experiment where pigs (gilts and castrates) were grouped based on a high or low SGE for growth was conducted, to investigate underlying behavioural differences and confirm previous results (N=480).

Results: Aggression measured by skin lesions and fighting during regrouping did not differ between high and low SGE pigs. However, pigs with a high SGE showed less aggression after reunion with familiar pigs and also had less non-reciprocal biting in the week after regrouping. During the finishing phase, high SGE pigs showed systematically less biting behaviour; 40% less aggressive biting and 27% less oral manipulation of pen mates. High SGE pigs were also chewing 40% less on distraction material and consumed 30% less of the jute sacks provided. These differences were also expressed in the tail damage, where high SGE pigs had a better tail score (less damage) compared to low SGE pigs.
One remark on this one-generation selection experiment should be made. The contrast on growth, by selecting sires and dams with high and low SGEs, did not result in significant differences in growth in their offspring.
Conclusion: Social genetic effects offer the opportunity to breed for improved behaviour while maintaining performance, although results are sometimes conflicting and accurate pen registration is essential. Since group housing is standard practice in finishing pigs, the pen is the production unit and the pen is of great importance for the production, welfare and health of the pig.

Disclosure of Interest: None Declared

Keywords: agression, genetics, welfare

Oral Abstracts - Thursday 09 June 2016

O-WN1-003

Organic enrichment material in pig farming: A hygienic risk?

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Introduction: Based on the Council Directive 2008/120/EC, permanent access to enrichment material, ideally organic material, for pigs has to be provided. According to an EFSA Scientific Opinion, these materials may pose risks, and further research is needed. This study aims at the hygienic status of different organic enrichment materials and to evaluate possible risks of pathogen introduction.
Materials and Methods: Twenty-one organic materials were examined; 18 were commercially available in Germany, three (straw, hay and silage) were produced on-farm. Four materials were made of wood; eleven consisted of partly compressed straw or hay. The remaining uncategorised six materials were: beet pulp, maize pellets, peat, licking blocks for pigs, litter made of lignocellulose, and maize silage. The weighted samples were grinded, and a suspension was prepared for microbiological examination. All materials were tested for total viable count (TVC), coliform count, *Escherichia coli* (*E. coli*), *Klebsiella* spp., *Yersinia* spp., *Salmonella* spp., fungi, methicillin resistant *Staphylococcus aureus* (MRSA), and *Mycobacterium* spp.. In addition, a high-performance liquid chromatography-mass spectrometry based multi-mycotoxin analysis was performed.
Results: The TVC ranged from 0 colony forming units per g dry matter (cfu/g DM) (wood shavings, beet pulp, lignocellulose litter) to 7.7 x 10^7 cfu/g DM (maize silage). *E. coli*, *Klebsiella* spp., *Salmonella* spp. and MRSA were not found in any of the tested materials. In a litter material made of hemp, *Mycobacterium smegmatis* was detected. The wood and compressed straw and hay products showed a microbial load of 10^3 to 10^7 cfu/g DM. In the loose straw and hay products a higher microbial load with 10^5 to 10^7 cfu/g DM was detected. Peat showed a comparatively high TVC and fungi count (4.5 x 10^6 and 2.2 x 10^5 cfu/g DM, respectively), but coliforms were not detected. The analysis of mycotoxins revealed a high mycotoxin load in some materials. For instance, the pelleted maize contained 47 of 380 tested mycotoxins, and 5,322 µg/kg deoxynivalenol and 1,285 µg/kg zearalenone.
Conclusion: The tested materials differed in their hygienic status widely. Important pathogens such as *E. coli* or *Klebsiella* spp., or zoonotic agents such as MRSA or *Salmonella* spp. were not found in any material. Some materials, especially maize products, contained high amounts of mycotoxins which pose a health risk for pigs. In conclusion, not all tested materials are suitable as enrichment materials for pigs. In the next steps, tests for viruses and tenacity as well as on-farm tests will be established. This study is supported by H. Wilhelm Schaumann Stiftung and Tierseuchenkasse Niedersachsen.

Disclosure of Interest: None Declared

Keywords: biosecurity, environmental enrichment, pathogen introduction

O-WN1-004

Studies on heritability of androstenone and skatole content in neck fat, sensory evaluation of boar taint and performance of male and female fatteners

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Introduction: Due to new legislation, fattening of uncastrated male pigs is an important option for future pork production. Beside advantages boar taint – mainly caused by androstenone (A) and skatole (S) in fatty tissue - may result in objection of the carcass. This study was performed in order to generate solutions by breeding
Materials and Methods: In the first step male pigs (terminal sire lines: Pietrain db.06; n=617 from 28 sires; Duroc db.08; n=127 from 8 sires) from a boar rearing farm were selected, slaughtered and neck fat samples were analyzed for A (ELISA) and S (HPLC). Heritability was calculated with VCE6.0. In the next step 12 defined sires were allotted to 4 genetic groups (high vs. low in A/S; A+A-; S+S-). Sows were paired with these sires to produce in total 1440 male/female fatteners. Average daily weight gain (ADG; mean individual weight at start: 27.2 kg; before slaughter: 124.6 kg), carcass characteristics (by AutoFOM) and neck fat samples (A and S content) were determined. Sensory evaluation on boar taint was done on 180 randomized samples (evenly distributed for genetic groups) by a panel of trained assessors. Alternatively, samples were regarded at risk for decreased consumer acceptance using “thresholds” for A (2.0 µg/g) and for S (150 ng/g fat). Statistical analysis was performed by procedure mixed (SAS 9.1). Differences between LS-means were tested by Scheffe-test $p<0.05$.
Results: Estimated heritability was .51 for S and .49 for A. Overall 96.6% of Pietrain and 63.8% of Duroc male fatteners did not exceed thresholds for A and S. In the field trial contents of A and S (ng/g) in neck fat of males differed as expected between the groups (A+S+: A 1449, S 111; A-S+: A 1009, S 92; A+S-: A 1336, S 84; A-S-: A 854, S 72). Values for sows were distinctly lower similar for all genetic groups (ng/g fat means of all groups: A: 90-148; S: 29-34). The ADG of males (means of groups 863-882 g/d) were significantly higher than of females (means of groups 829-835 g/d). No differences were found between genetic groups. Carcass yield was significantly lower in boars (means of groups: 76.0-77.1%) than in sows (means of groups: 79.1-79.7%). Ham (H) and loin (L) weights (kg) were higher for sows (means of all groups H 18.4-18.6; L 7.23-7.34) than for boars (means of all groups: H 18.0-18.2; L 6.99-7.14). Results of sensory testing were in good agreement to analyzed levels of A and S in neck fat.
Conclusion: The estimated heritabilities of A and S allow breeding against boar taint. Results obtained confirm that performance is not necessarily reduced. The sensory test of neck fat on boar taint was in good accordance with the analyzed contents of A and S.

Disclosure of Interest: None Declared

Keywords: boar taint, breeding, performance

O-WN1-005

Stunning and killing of non-viable piglets under the aspect of animal welfare - training with a piglet simulator

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Introduction: In Lower Saxony a new regulation for stunning and killing of non-viable piglets up to a weight of 5 kgs came into effect in July 2014.
In addition to the expertise, the practical experience of the farmers and vets is required now.
The demand of training courses was high, so the Animal Health Service in the Chamber of Agriculture had decided to offer training modules.
In accordance with the Ministry of Agriculture and the Office of Consumer Protection and Food Safety, training materials were created.
The Clinical Skills Lab at the University of Veterinary Medicine Hannover developed a silicon piglet simulator. The simulator allows practical exercises to avoid the killing of piglets during the training of stunning and killing.
Materials and Methods: Based on the corpse of a dead piglet, the Clinical Skills Lab developed a mold that enabled the repeatable production of a silicone piglet body of correct anatomically structures and size.
The size of the piglet simulator, the locations of the occipital bone, the sternum and the limbs were exactly modeled to simulate the body´s form of a suckling piglet.
The fidelity of the simulator focused to simulate the correct fixation and the impact of the head at the right position to quickly stun the animal.
In accordance to the vets of the Animal Health Service and the Clinic for Swine at the University of Veterinary Medicine Hannover, silicone tubes were installed into the throat region, to simulate the arteriae caroticae.
Red liquid was added into these vessels, so during a cut through the throat, “bleeding” can be simulated.
The result is a combination of the practical exercises on the piglet simulator, and the teaching materials, in which the the accordance to current law, the various ways of anesthesia and killing are explained.
Within this training course, the correct fixation of the animal, the “impact of the head anesthesia” and the killing with a cut through the throat can be practiced on the piglet simulator without violating or killing alive animals.
Results: The combination of learning resources - which are mediated by PowerPoint presentations and videos - and the lifelike piglet simulator is a successful method of practical training according to the animal welfare.
Because of the realistic looking piglet and the simulated "bleeding" the student or the stockman are able to locate anatomical points and to find the right way to cut into a body without harming an animal alive.

Conclusion: To ensure the animal welfare future simulators will be developed to train vets, farmers and students.
This will be simulator piglets and adult pigs for blood sampling, lung lavage removals, anesthesia, hoof care and obstetrics.

Disclosure of Interest: None Declared

Keywords: Killing and Stunning, piglet Simulator

O-WN2-006

Herd level risk factors for stomach ulcers in finishing pigs

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Introduction: The influence of feed structure and pelleting on the development of gastric ulcers in pigs is well established. Some studies have shown a beneficial effect of access to straw on gastric health. However, the role of other factors, e.g. disease and stress, is not well understood. The aim of this study was to identify herd factors associated with a high prevalence of gastric ulcers in finishing pigs. Based on results from earlier studies, it was decided to focus on feed type, infectious diseases, the use of straw and the floor type in the pens.
Materials and Methods: The managers of 37 Danish finishing farms answered a questionnaire about housing, feed, health and management on their farms. The farms were originally selected for a study on mortality, and they all had either a very low (21 farms) or a very high mortality (16 farms). The stomachs of 20 pigs per farm were examined macroscopically at slaughter, and pathological changes in the oesophageal part of the stomach were scored on a scale from 0 to 10 (score 0: no changes, score 1-5: parakeratosis/erosion, score 6-10: ulcer/fibrosis).
The association between the risk of having a stomach score of 6-10 and potential risk factors at the herd level was analysed by logistic regression analysis (PROC LOGISTIC, SAS). The explanatory factors were: feed type (pelleted/meal), herd health status with regard to infection with *Actinobacillus pleuropneumoniae* serotypes 2 and 6 (*App* 2, *App* 6) (yes/no), PCV2-vaccinated pigs (yes/no), access to straw in a straw rack or on the floor (yes/no) and type of pen floor (percentage of slatted floor, analysed as a continuous variable). In addition, mortality level (low/high) was included as an explanatory factor in the final statistical model.
Results: The prevalence of stomachs with a score of 6-10 was 51% in herds with low mortality and 62% in herds with high mortality, but the difference was not statistically significant. The use of pelleted feed (OR=3.7), PCV2-vaccinated pigs (OR=1.9) and the herd infection with *App* 6 (OR=1.6) were associated with an increased risk of a stomach score of 6-10 ($p<0.05$). No effects of *App* 2 infection, straw or floor type were found.
Conclusion: The study confirmed the negative effect of pelleted feed. PCV2-vaccinated pigs had an increased risk of having stomach ulcers, probably because it is more common to PCV2-vaccinate in herds with stomach ulcer problems. The association between *App* 6 infection and stomach ulcers supports the results of an earlier Danish study, in which an association between pleurisy and stomach ulcers was found. This study included only a limited number of herds, and only substantial differences would be detected.

Disclosure of Interest: None Declared

Keywords: Feed, Gastric ulcers, Risk factors

Oral Abstracts - Thursday 09 June 2016

O-WN2-007

Influence of gestation housing system on sow health and the transfer of maternal immunity to the neonate

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Introduction: Although the conventional housing on slatted floor remains predominant in European pig farms, a variety of alternative housing systems exists for gestating sows. The consequences of these different environments on health of the mothers and on the immunity they transfer to their progeny remain poorly known. This study aimed at determining the influence of two contrasted housing systems during gestation on welfare and health traits of gestating sows, as well as on the cellular and humoral immunity transferred to the neonate through mammary secretions.
Materials and Methods: Gestating sows were raised in groups of 24 individuals in a conventional system on slat (C, n=18) or in larger pens enriched with straw bedding (E, n=19). Approximately 10 days before farrowing (gestation day (DG) 105), sows from both systems were transferred to farrowing units with similar conventional crates. Lameness of sows was assessed during the transfer. Saliva was collected for cortisol assay on the morning of DG 35 and 105. Blood was collected on DG 105 for leukocyte count, haptoglobin, oxidative stress, immunoglobulin (Ig) G and A measurements. Mammary secretions were collected at farrowing (1-2 h after the birth of the first piglet) and 4 days later (L4, milk collection after 1 ml oxytocin administration) for cell numeration, and IgG and IgA content analysis. On L4, two piglets per litter were blood sampled for IgG measurement.
Results: Salivary cortisol concentration was lower in E than C sows at both DG35 and 105 ($P < 0.001$). At DG105, E sows had lower blood granulocyte counts (-17%, $P < 0.001$) and hydroperoxyde concentration (-19%, $P < 0.01$). The biological antioxidant potential, haptoglobin, IgG and IgA concentrations did not differ ($P > 0.10$) between the two groups of sows. At the transfer to farrowing stalls, lameness was significantly more prevalent in C than E sows (18 vs 2%, $P < 0.001$). The absolute numbers of total cells, and among them of immune CD45+ cells, per ml of mammary secretion were similar in E and C sows in colostrum but greater in E than C sows at L4 (+125%, $P < 0.05$). Concentrations of IgG in colostrum, IgA in milk at L4, and IgG in piglet blood at L4 were similar in C and E animals.
Conclusion: To conclude, cortisol, lameness frequency, granulocyte counts and oxidative stress markers indicated that health and welfare of sows were greater in the E system. These differences during gestation did not affect the transfer of cellular and humoral immunity to the piglets via colostrum, but might have affected the transfer of cellular immune components in the milk afterwards. Research has received funding from the EU FP7 Prohealth project (no. 613574).

Disclosure of Interest: None Declared

Keywords: housing, Sow, welfare

O-WN2-008

On-farm tail biting prevention in long-tailed pigs – results from a producer questionnaire in Finland

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Introduction: Tail biting is a serious welfare problem in pigs, causing substantial economic losses. In the majority of the EU countries, tail docking is used to reduce the incidence of tail biting. However, many of the risk factors for tail biting are related to suboptimal management, and tail biting can be reduced by corrective management decisions. There are few studies on which preventive measures producers themselves value as most important.
Materials and Methods: A questionnaire was distributed via slaughterhouse webpages in 2015. Producers were asked to score the importance of handling different tail-biting risk factors on their own farms, as well as about which manipulable materials they use, and find efficient. In addition, we asked about their opinions on tail biting and tail docking. A total of 70 producers replied, 54 of these replies were regarding fattening pigs, and 16 regarding weaned pigs. The size of the pig units varied between 100 and 6400 pigs, with an average of 1307 pigs. Finland banned tail docking in 2003, so all farms raised long-tailed pigs only.
Results: On average, the producers reported a prevalence of tail biting of 2,3% on their farms, which corresponds well with values reported at Finnish abattoirs. Most producers found tail biting not to be a big problem on their farms and 62% of the farmers found it very unlikely that they would raise tail docked pigs even if it was legal in Finland. The more tail biting reported on the farm, the more problematic the farmers found tail biting, and the more prone they were to say they would probably tail dock if they were allowed to.
According to the Finnish producers, the most important factor to prevent tail biting is that there is enough feeding space for the pigs. Altogether, four feeding-related risk factors were included in the top-10 measures to prevent tail biting. Also pig health was considered very important, as well as a good quality of piglets, and controlling air movements in the pen. Straw, newspaper, hay and cardboard were considered the most efficient manipulable materials to prevent tail biting. If tail biting has already started in the pen, the producers ranked identifying and removing the tail biter from the pen as most important, followed by adding bedding-type manipulable materials.
Conclusion: The results are partly in accordance with experimental and epidemiological studies on risk factors for tail biting, but the high focus on feeding-related and health factors is interesting. Finnish farmers appear to handle the tail docking ban well, and do not, on average, find tail biting a very serious problem.

Disclosure of Interest: None Declared

Keywords: Producer attitudes, Tail biting, Tail docking

O-WN2-009

Virtual farrowing unit - Increase piglet survival in a game-based setting

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Introduction: Incorrect procedures performed by farm workers or veterinarians can be costly and may jeopardize animal welfare. In the education of both veterinary students and farm workers, practical training is sparse and hands-on clinical skills can be difficult to obtain. Game-based virtual training facilities are interactive and require participation as well as student decisions. Virtual herd visits allow students to practice diagnostic, communicative and interdisciplinary skills in a safe environment. Motivation is secured by use of points, different game scenarios and integration of gaming activities. The combination of interactive knowledge acquisition and motivational factors aim to exploit the learning potential of game based learning.
Materials and Methods: Animal welfare was the overall theme for the first of several game based modules. Piglet survival rate and compliance with Danish legislation were key elements in the first module: "Farrowing unit". Game design and content were described in cooperation between Danish agricultural colleges, University of Copenhagen, pig practitioners, veterinary officers, game developers and didactic specialists. To increase piglet survival, three key areas related to management were identified: correct farrowing assistance, identification and correct treatment of sows with farrowing fever and insurance of an optimal environment in piglet nesting area.
Video clips and photographs were collected in pig practice. Golden standards according to Danish legislation were derived from the self-audit scheme provided by the Danish Pig Research Centre.
Results: Game design uses a mix of animation and real photos/videos. Students engage in playing as the newly employed farm worker, responsible for the farrowing unit. Upon entrance into the farrowing unit, the manager of the farm introduces the player (student) to tasks related to the unit. Subsequently, the player must plan tasks in a logical order. The tasks include: farrowing assistance, allocation of nest building material, training of piglets to use nesting area hereby insuring an optimal piglet environment , iron injection, castration, tail cutting and finally a replacement sow must be chosen as too many piglets are live born. All tasks must be performed correctly and in the right order. The point system is a dynamic piglet survival barometer where mistakes cause piglet survival rate to decrease.
Conclusion: The game provides a virtual farrowing unit where students must communicate with other farm personal, keep track of time and procedures and perform correctly. By adding new tasks during the game and by the stress caused by the piglet survival rate barometer, players will stay motivated and challenged throughout the game.

Disclosure of Interest: None Declared

Keywords: Animal welfare, Game-based learning, Piglet survival

O-WN3-010

Systematic review and meta-analysis on the effect of xylanase supplementation on average daily gain in pigs.

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Introduction: Xylanase is an enzyme which is supplemented in monogastric diets to increase non-starch polysaccharide digestibility. Inconsistent results are found in the literature when diets for growing-finishing pigs are supplemented with xylanase. Xylanse has been supplemented in diets varying in ingredient content and nutrient composition. The aim of this study is to explore the optimum dietary characteristics of xylanase supplementation in pigs.
Materials and Methods: A literature search was conducted for articles published between 1990 and 2015 using the database Web of Science with the search terms: "xylanase" and "growth" and ("pig" or "piglet"). From 153 articles found and after deletion of articles not fulfilling the requirements to be included in the analysis, 22 articles were selected and data from 43 trials was recorded. Each study was assigned to a category depending on (1) diet type: European (if the % of wheat and/or barley was >60%), American (if the % of maize was >40%) and By-products (if the % of by-products was >20%); (2) energy density: higher or lower than the DE levels recommended by NRC (2012); (3) growing phase (weaning, growing or finishing). A meta-analysis was carried out to summarize the effect size of xylanase supplementation on average daily gain (ADG, g/day) using the metafor package in R. Mean difference (MD) was the effect size estimate, calculated by subtracting the mean ADG of the control group from the supplemented group, the pooled SEM was used as sampling error. Growing phase, diet type, energy density, sex, xylanase dose, percentage of crude protein and neutral detergent fibre content in the diet were tested individually in a univariate analysis. The variables that had a $P<0.25$ in the univariate analysis were included in the final multivariate model.
Results: The variables included in the multivariate model were diet type ($P<0.05$) and energy density ($P<0.05$). The result of the multivariate analysis showed a benefit to supplementing diets with xylanase of 29 g/day ($P<0.01$) with a confidence interval from 6.0 to 51.5 g/day. American diets based on maize had a higher ADG response than European diets based on wheat and barley. However, fewer experiments using maize were reported and there was less variation in the response between these studies than was found for the European diets. Low energy density diets had a higher ADG response than diets formulated to exceed NRC requirements.
Conclusion: Dietary ingredient composition and energy density affect the growth response observed when xylanase is supplemented to the diets of growing pigs. A positive growth response to xylanase can be expected when supplemented to diets which are formulated below the NRC recommendations for DE.

Disclosure of Interest: None Declared

Keywords: Growing-finishing pigs, meta-analysis, xylanase

Oral Abstracts - Thursday 09 June 2016

O-WN3-011

Return to oestrus caused by impaired feed intake; a case report

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Introduction: Chronic physical stress and feed deprivation attribute to the risk of return to oestrus in mammals. Some of these factors have been shown in pigs experimentally, but case reports are rare. Data from electronic sow feeders (ESF) enable analysis on the association between feed intake and reproduction results. This case report describes the results of such analysis in a 1025 head sow farm which was experiencing a slight increased return to oestrus (8.8%) and a lower farrowing rate (86.5%) in 2014 compared to Dutch average figures. Return to oestrus peaked in gilts (20.4%) with a median interval between insemination and return to oestrus of 33 d (range 19 - > 60 d) leading to a probable diagnosis of early embryonic death as a cause of return to oestrus in gilts.

Materials and Methods: Risk factors for chronic stress, such as feeding, housing, and management were thoroughly evaluated. Feed content, feed intake and reproduction data of 4 week groups (n= 220 sows) were analysed for associations with return to oestrus. Impaired feed intake was defined as at least two days with more than 70% rest feed in the first 14 days after introduction in the group housing.

Results: Gestating sows were housed in 4 dynamic groups of 200 sows with 4 electronic sow feeders per group and straw bedding in the resting area. One group was used for parity 0 and 1 sows (p0 &p1).

Whereas analysis of feed content showed adequate nutrient levels, intake data showed that 82% of p0 and 27% of p1 had more than 70% rest feed at least twice in the first two weeks after introduction in the group housing in contrast to 5.7% in p≥1 sows. 11/43 p0 and 3/44 p1 returned to oestrus. None of p0 without rest feed returned to oestrus. Cox proportional hazard analysis, contrasting impaired feed intake (Y/N) for p0 and p1 sows to the p>1 sows, showed a hazard ratio of 2.8-5.9 for return to oestrus in the impaired feed intake group.

Analysis of ESF gilt training showed several flaws. Multiple stressors were found attributing to deprived learning. Firstly, gilts were regrouped and moved thrice after arrival on the farm. Aerial ammonia was 40 ppm and gilt-human interaction was inadequate. Most importantly, in the training area a through with ad lib feed was provided behind the training ESF. Gilts were thus conditioned to transit through the ESF to the through, instead of learning to eat in the ESF.

Conclusion: This case report shows that combined analysis of data on feed intake and reproduction can be of additional value in the field and clearly pointed to the impact of improper training of gilts to eat from a ESF.

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Disclosure of Interest: None Declared

Keywords: Electronic sow feeding, Impaired feed intake, Return to oestrus

O-WN3-012

Effect of antioxidant supplementation and heat stress in a model of double vaccination in weaned piglets.

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Introduction: Piglet weaning is an interesting model of oxidative stress (OS), but due to large inter-individual variations, there is a need to standardize *in vivo* response by applying usual challenges, such as vaccination (vacc) and heat stress (HS). The objectives of this trial are the development of a reproducible model of OS and the determination of the most accurate blood biomarkers. The effect of a combination of antioxidants was also investigated.
Materials and Methods: 360 starter pigs were randomly affected to eight groups in a 2×2×2 factorial design. Trial started at weaning (D0) and was divided into 2 phases: phase 1 (D0-14) and phase 2 (D14-41). Double vacc at D0 against PCV2 and porcine influenza, HS at days 9-10, 23-24 and 37-38 (37°C over 2×6h periods), or controls, were applied to piglets which were given during phase 1 NRC (2012) levels for vitamin E and selenium (low) or extra supplementation in vitamin E, selenium and SOD-rich melon pulp concentrate (high). Piglets were weighed at D0, D14, D28 and D41 and average daily feed intake (ADFI) and average daily gain (ADG) were measured for each phase. Blood was sampled at D13 and D40 and analyzed for GPx activity, lipid peroxides (LP), haptoglobin (HP) and half-haemolysis time (T1/2) of whole blood (WB) and red blood cells (RBC).
Results: Double vacc impacts negatively ADFI (-3%) in phase 2 and ADG in post-weaning (p=0.03). Vacc negative effect was suppressed when piglets were supplemented with antioxidants: vacc piglets had lower ADG in phase 2 than non-vacc piglets in the low group (564 vs. 591 g/day, p=0.03), while there was no vacc effect in piglets for the high group (577 vs. 575 g/day for non-vacc and vacc piglets, respectively). Conversely, HS had worsening effect on performance in phase 1: 222 and 210 g/day for vacc piglets without and with HS, respectively (p<0.05). Vacc increased inflammatory status (increased HP, p<0.05) and OS (decreased plasma GPx activity and increased LP, p<0.05) at D13 and D40, while antioxidants decreased OS (increased plasma GPx activity, decreased LP and increased T1/2 of WB and RBC, p<0.05). Moreover, at D40 HS had negative effect on T1/2 of WB and RBC in vacc piglets (p<0.01) and additive negative effects of HS and vacc were observed on LP.
Conclusion: Combining a double vacc against PCV2 and porcine influenza with repeated periods of HS appears efficient to develop an accurate model of OS in weaned piglet. Vacc has adverse effects on performance and increases OS and inflammatory status. HS appears here as a worsening factor of vacc, while combined antioxidants (vit E, organic selenium, SOD) helps restoring antioxidant status and zootechnical performance.

Disclosure of Interest: None Declared

Keywords: vaccination, antioxidant, heat stress

O-WN3-015

Effects of a high fibre diet around parturition in combination with an ad libitum feeding regime on the performance of sows and piglets

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Introduction: Over the last few years, the number of born and weaned piglets per sow has been increased continuously. The aim of this study was to investigate, whether a separate allocation of a high fiber diet in addition to an ad libitum feeding of sows in farrowing pens, leads to beneficial effects for sows and piglets.
Materials and Methods: From day 109 (d-7) of gestation, a total of 34 sows were fed daily two portions of a commercial lactation diet (per kg DM: 190g XP, 48.3g XF, 14.7 MJ ME) until d35 of lactation following a manually controlled restricted feeding scheme. 12 sows (ADLIB group) had the possibility to get additional feed out of a feeding dispenser containing from d-7 until d2 a fibre-lactation-diet mixture (~85 % fibre pellet – per kg DM: 125g XP, 179g XF, 8.85 MJ ME - and ~15% lactation diet) and from d3 onwards lactation diet. Feed intake of sows was measured daily. Furthermore faecal samples of all sows were taken. On d-7 and d35 body weight and back fat thickness of each sow were determined. Within 24h after parturition of the last sow, litters were standardized (ADLIB n=13.7±0.89, CONTROL n=13.6±1.05) by cross-fostering piglets; each piglet was weighed weekly. Differences between the groups were tested using the t-test (normal distributed) and the Wilcoxon-test (not normal distributed data; significance level: $p<0.05$).
Results: The voluntary feed intake in DM of the fibre-lactation-diet mixture a. p. was 3.14±0.68kg. The ADLIB fed group had a higher DM intake in lactation (7.37^{a}±0.67kg vs. 6.69^{b}±0.50kg) and lower body weight losses (-29.5^{a}±14.2kg vs. -40.3^{b}±13.5kg). Both, the loss of back fat thickness was in tendency lower (-2.39±2.15mm vs. -3.63±1.98mm) and the absolute piglet's weight gain was slightly higher in the ADLIB group (122±13.0kg vs. 117±20.2kg). The number of raised piglets (12.8±1.49 vs. 12.8±2.05) did not differ between the groups. The dry matter content (DM in % on d-2: 26.4^{a}±2.51 vs. 29.6^{b}±4.08, d1: 30.4^{a}±4.42 vs. 33.3^{b}±4.67, d3: 26.6^{a}±4.30 vs. 29.1^{b}±3.92) and pH in faecal samples (pH on d-2: 6.30^{a}±0.18 vs. 7.18^{b}±0.38, d1: 6.35^{a}±0.53 vs. 7.15^{b}±0.35, d3: 6.49^{a}±0.33 vs. 7.04^{b}±0.40) were significantly lower in the ADLIB group. Birth interval between two piglets was slightly reduced in the ADLIB group (12.0±4.32min vs. 13.4±5.05min).
Conclusion: A higher feed intake a. p. does not seem to interfere with the farrowing process. Although an increased fibre intake around parturition leads to softer faeces and reduced pH, duration of farrowing is not reduced significantly. An ad libitum feeding regime seems to have beneficial effects on feed intake in lactation and on mobilization of body fat reserves.

Disclosure of Interest: None Declared

Keywords: ad libitum feeding, high fibre diet, lactation sow

Oral Abstracts - Thursday 09 June 2016

O-HHM1-004

Estimating the costs of Porcine Reproductive & Respiratory Syndrome (PRRS) at individual farm level using a tailor-made mathematical model.

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) is among the diseases with the highest economic impact in pig production worldwide. Losses due to the disease were estimated as high as 560 Mio. US$ per year in the USA. Yet, the economic impact of the disease at farm level is not well understood as, especially in pig herds chronically infected with PRRS virus, the losses caused are often not obvious for farmers and veterinarians Thus, the aim of this study was to develop an epidemiological and economic model to determine the costs of PRRS for an individual pig farm.
Materials and Methods: The model developed by Alarcon and co-workers to estimate costs of *Porcine circovirus type 2 associated diseases* in pig farms was used as a basis for modelling PRRS infection and costs in pig farms. In a production model that simulates the production of different farm types, batch systems, etc. - to account for different industry settings frequently found in Germany, Spain and The Netherlands - an epidemiological model was integrated. In this, the impact of PRRS infection on health and productivity, depending on PRRS severity, is estimated. From this, financial losses are calculated in a gross margin analysis and a partial budget analysis. Data on the effects of chronic infection and a disease outbreak on reproductive performance, piglet morbidity and mortality, daily weight gain, feed efficiency and treatment costs were obtained from the literature, from industry databases and from results of field studies, whenever possible. Data that were unavailable were estimated based on expert opinion.
Results: The final calculator was coded as a spread sheet model in Microsoft Excel and displays the economic effect of PRRS at individual farm level. The model can account for different farm types, i.e. piglet producing farm, nursery farm, fattening farm and farrow-to-finish farm, for different herd sizes, types of batch farrowing (one-week- and three-week-rhythm), lengths of suckling period (three, four and five weeks), etc. The model input can be customized by front-end users with their particular farm characteristics. As an output, the user receives the marginal costs due to PRRS per sow or farm per year, a separate demonstration of single costs due to PRRS on his/her farm and the percentage of profits lost due to the disease.
Conclusion: The model is a valuable tool for farmers in recognizing the economic impact of PRRS on their own pig farm. The output can help to understand the need for interventions in case of significant impact on the profitability of their enterprise. The model can support veterinarians in their communication to farmers in cases where costly disease control measures are justified.

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Keywords: Economic impact, Farm level calculator, PRRS

Herd Health Management and Economy

O-HHM1-002

Animal level risk factors associated with reduced growth from birth to slaughter

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Introduction: Slow growing pigs can be a limiting factor for all in - all out production and they can reduce the farmer's income. A 10% reduction in ADG from weaning to slaughter will reduce the gross margin per pig by 2.5 euro. The objective of this study was to identify risk factors and estimate their impact on growth rate on population level.
Materials and Methods: The hypothesis was that the duration of the period that a sow entered farrowing unit before farrowing, parity, litter size, gestation period, night and weekend farrowings, back fat of sow, assisted birth, use of oxytocin, infections in sows, piglet gender and birth weight, cross fostering, movement, and infections in pigs are associated with reduced growth.
The study was performed as a cohort study in 9 farrow to finish herds. Approximately 70 consecutive farrowings in each herd were included in the study. For sows their ID, parity, farrowing data, and treatments were recorded. Each piglet was weighed and ear tagged at birth. All pig treatments, movements and deaths were recorded.
Pigs growing < 569 g/day were defined as slow growing pigs (25% slowest growing in the data set).To identify risk factors for being a slow growing pig univariate analysis was performed by logistic regression on piglet level. Factors significant at P < 20% were included in the multivariate analysis. To estimate the impact on population level the Population Attributable Risk (PAR) was estimated by a macro that included all the significant risk factors from the multivariate analysis (P < 5%). The reduction in ADG on pig level was estimated by linear regression.
Results: A total of 6071 live born piglets were included in the statistical analysis. The multivariate analysis showed that birth weight < 1kg (Odds Ratio (OR) 2.7) , parity < 4 (OR 1.2), total born > 18 (OR 1.2), movement of pigs in farrowing units (OR 1.4), female pigs (OR 1.2), treatment of suckling (OR 1.4) and nursery (OR 1.5) pigs were associated with slow growth. The estimates of PAR showed that without the risk from pigs with birth weight < 1 kg the proportion of slow growing pigs could be reduced by 18%. Without the risk from parity < 4, total born> 18, movement of pigs in farrowing units, female pigs, treatment of suckling and nursery pigs the reduction could be 11%, 9%, 9%, 8%, 3%, and 4%, respectively. The corresponding reductions in ADG per pig were 44, 8, 8, 6, 8, 14, and 22 g/day, respectively.
Conclusion: This study indicates that the potential proportion for reduction in slow growing pigs was 48% by focusing on the identified risk factors. A pig with all the identified risk factors would grow 110 g/day less from birth to slaughter.
Disclosure of Interest: None Declared
Keywords: Population Attributable Risk (PAR), Reduced growth, Risk factors

Herd Health Management and Economy

O-HHM1-005

Development and validation of a model for generating disease detection sample sizes where oral fluid and other pen-level sampling methods are used.

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Introduction: The methods for determining the number of individual animal samples to collect for use in the detection of disease agents in populations has been well described and is relatively well understood among veterinarians. However, with an ever increasing number of samples being collected using pen-level sampling methods (e.g., oral fluids) it is important for veterinarians to understand the methods for and have access to tools designed to determine appropriate pen-level sample sizes. In contrast to individual animal detection sampling, pen-level sampling is a function of both the population of animals and number of pens, the disease prevalence of individual animals as well as pens, and the desired confidence level for detecting one or more positive pens (not animals). A model were developed and validated to enable determination of appropriate pen-level sample sizes for disease detection.
Materials and Methods: Basic model user-defined input variables are: animal population size, number of pens, animal prevalence, pen prevalence and desired pen-level confidence. To account for the influence of within-pen animal prevalence on pen-level prevalence, an adjusted pen prevalence is calculated. Within-pen animal prevalence of pens containing positive animals are model input variables. The within positive pen animal prevalence and a binomial distribution were used for estimation of the number of positive animals in positive pens. Where the model generated pens containing more than one positive pig the pen-level detection probability was determined by accounting for the incremental detection probability attributable to each additional positive animal. Pen-level sampling model inputs assumptions were: 1000 animals, 40 pens, 95% detection confidence, and 50% pen-sample detection probability for each positive animal in a positive pen. Animal/pen prevalence levels evaluated were: 3%/10%, 5%/20%, 10%/30%, 15%/40% and 20%/50%. To validate pen-level sampling model results, a second (stochastic sampling simulation) model was used to generate 1000 sets of 10 replicates per set.
Results: For the animal/pen prevalences listed above, the model estimated that 21, 12, 8, 6 and 5 of 40 pens should be sampled, respectively. For those sample sizes (21, 12, 8, 6 and 5) the validation model estimated the detection confidence/pen prevalence at 93.6%/11.4%, 94.0%/20.6%, 93.7%/29.5%, 95.1%/38.5% and 96.8%/49.4%, respectively.
Conclusion: This novel pen-level sampling model can be used to dynamically estimate the appropriate number of pen-level samples to collect for use in disease detection, as well as generate tables to be used as references for pen-level detection sampling.

Disclosure of Interest: None Declared

Keywords: None

Herd Health Management and Economy

O-HHM1-001

Effect of pig company size on production parameters and pig production cost form 2010 -2014 in Spain.

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Introduction: Economies of scale are the cost advantages that enterprises obtain due to size, output, or scale of operation, with cost per unit of output generally decreasing with increasing scale as fixed costs are spread out over more units of output. Pig size company is an overlooked factor to explain the variability observed in production parameters and pig production cost between pig production enterprises. The goal of the present work was to describe the effect of pig company size on production parameters and pig production cost from 2010 to 2014 in Spain.

Materials and Methods: Between 61 and 107 pig production companies from Spain were included in this study from 2010 to 2014. These companies were operating in farrow-to-finish, two-site and three-site production systems and they were classified in four groups according to the number of sows in operation: <1000, 1000-5000, 5000-1000 and >10000 sows. These companies sent data on feed consumption, number of pig produced, expenses and census every month. Sip consultors SL standardized collected data and calculate cost and production parameters to obtain values comparables between the different pig production companies. The collected data each month were merged to obtain a yearly average value taking into account the pig production flow each month. A statistics descriptive was calculated for each parameter during the period 2010 to 2014. An Anova or Wilcoxon test was used to analyse the association between continuous normally or non-normally distributed variables and the pig size company.
Results: In general terms, the best technical parameters during the piglet, nursery and fattening production phase were obtained for companies with less than 5000 sows. However, the bigger the pig size company is, the lower the feed price is for all the production phases. Pig company size affected most of the pig production cost determinants. Thus, the lower the pig size company is, the lower total drug and vaccine cost, total fixed cost and total reproduction cost per pig are. However, the opposite tendency is observed for the total feed cost per pig where the highest value was observed for the smallest companies. Globally, total cost per produced Kilogram is very similar between companies with the exception of companies between 5000 and 10.000 sows whose value is higher than for the rest of companies.
Conclusion: Pig company size is affecting not only production parameters but also pig production cost for companies with a sow number between 5.000 and 10.000 and it seems that this pig company size does not have any advantage in terms of profitability in the future.

Disclosure of Interest: None Declared

Keywords: pig company, production parameters, size

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Herd Health Management and Economy

O-HHM1-003

Pigs at risk: Impact of birth weight on piglet survivability

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Introduction: High prolificacy in sows and increased fetal survival leads to intra uterine crowding and growth retardation. As a result the piglet birth weight (BW) is decreasing and its variability increasing. Low BW results in higher mortality rates, reduced daily gain, reduced pork quality and increased feed conversion rate. Low BW has been defined as piglets beneath a certain weight cut off but there is no uniformity throughout the published studies.
The objective of this study was to define a criterion for low BW by investigating the relation between the individual BW of the piglet and its survival chances. Pigs below a predicted threshold are pigs at risk.
Materials and Methods: From 3 farms located in Spain 2331 piglets from 178 litters were followed from birth to slaughter or moment of death. Litter parameters were collected. If the pig died, the date, weight and cause were recorded. A mixed effects logistic regression model was fit to estimate the probability of pre-wean (PW) mortality based on BW. A piece-wise linear predictor was selected to best represent the drastic decrease in PW mortality found as BWs increase in the range of 0.5 to 1.0 kg and then the less extreme change in PW mortality observed for changes in weight above 1.0 kg. The change point of the linear predictor was found by computing the model fit for BW ranging from 0.5 to 2.0 kg and then selecting the point corresponding to the maximum likelihood for the model.
Results: The average litter size was 14.3 piglets of which 13.1 were live born. Average birth weight was 1.46 kg. Farm and litter size had an impact on birth weight. The total mortality was 17.5% with 14.2, 1.3, 1.1 and 0.9% of the pigs dying during lactation, nursery, growing and finishing phase respectively. 71.2% of the mortality occurred the first 5 days of life. Low viability and crushing were the main mortality reasons. There was a steep decrease in PW mortality as piglet BW increased from 0.5 to a change point at 1.13 kg, after which the mortality rate flattened out. Below 1.13 kg (17.5% of total) the chance of survival until weaning was 58%. Pigs from 1.13 kg up had a 92% chance of survival. After weaning the relation between birth weight and mortality disappeared.
Conclusion: Birth weight is a good predictor for survival chances of piglets independent of farm and litter size. There is a breaking point weight under which the survival chances are very low and the pigs are at risk. In this dataset, the breaking point was 1.13 kg. It would be very interesting to apply a similar approach to other datasets.

Disclosure of Interest: None Declared

Keywords: Birth weight, Survivability

Herd Health Management and Economy

O-HHM2-006

Health data management platform (HDMP) for disease surveillance, containment and elimination in a genetic multiplication system in North America

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Introduction: The goal of a breeding stock supplier is to consistently deliver genetic improvement to pork producers. To achieve this goal, a health assurance program is implemented to prevent the introduction of disease to the multiplication system and to customers' herds. The program is based on systematic risk assessment and mitigation, early detection of disease and opportune transparent communication among stakeholders. The objective of this abstract is to summarize the components of the HDMP and the results of its implementation in a multiplication system of 100.000 sows in North America.
Materials and Methods: The system includes 20 boar studs, 38 sow farms and 173 grow-finish sites. HDMP input data consists of (a) daily professional clinical observation, (b) monthly veterinary herd visits, (c) periodic farm biosecurity, feed mill and transport sanitation facility risk assessments, (d) routine and contingency diagnostics and (e) pig movements. Clinical and risk data is captured by a customized mobile application (*iAuditor – SafeCulture*) and transferred to the *PIC Health Hub* website for organization and analysis. Diagnostic results are generated daily from twelve veterinary diagnostic laboratories and consolidated by a real-time case aggregation tool (*LabLinkHIMS™ – Global Vet Link*). A collaborative effort between UC Davis, Iowa State University VDL, Boehringer Ingelheim Vetmedica Inc and Global Vet Link allows *Disease BioPortal* (*Center for Animal Disease Modeling and Surveillance – UC Davis*) to automatically receive input data and work as the analysis and display tool.
Results: During 2015, 1.200 veterinary herd visit reports, 120 farm biosecurity, 43 feed mill and 56 transport sanitation facility risk assessments were completed. Also, 8.700 diagnostic cases were submitted and 93.200 tests were performed. On 93 occasions a site was put on health-hold due to the detection of clinical signs, biosecurity breaches or unexpected diagnostic results. Six of those events resulted in specific disease introductions, two *M. hyopneumoniae* (*Mhp*) in sow farms and four PRRS cases in finisher sites. After more than 1.800 breeding stock deliveries, no evidence of specific disease introduction to customers' herds was reported. Two *Mhp* elimination projects were completed.
Conclusion: The HDMP has been instrumental in safely disseminating genetics from a large multiplication system with limited impact on supply and customer herds' health. The platform has allowed the close monitoring of health status and risk of disease introduction to individual sites on a real-time basis. Outbreak investigation, containment and elimination efforts have been supported by the information produced by the HDMP.

Disclosure of Interest: J. P. Cano Conflict with: PIC, M. J. Clavijo Conflict with: PIC, E. Spiekermeier Conflict with: PIC, V. Law Conflict with: PIC, J. Geiger Conflict with: PIC, J. W. Lyons Conflict with: PIC, T. Riek Conflict with: PIC, T. Snider Conflict with: PIC, R. Thompson Conflict with: PIC

Keywords: health management, risk assessment, Surveillance

Herd Health Management and Economy

O-HHM2-009

Seasonal variation in prevalence of different respiratory pathogens during post-weaning and fattening period in Benelux pig herds using TBS

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Introduction: Besides Mycoplasma hyopneumoniae (M.hyo), many other viruses and bacteria can be concurrently present during respiratory problems in pigs, provoking the disease complex known as Porcine Respiratory Disease Complex (PRDC). Diagnosis of infections with these pathogens can be performed using different approaches, including the detection of the pathogen through PCR assays. The sampling technique has been developed and validated for the detection of M.hyo in pigs using PCR, namely the tracheo-bronchial swab (TBS) technique. With this technique, pathogens present at the level of the trachea-bronchial junction can be recovered and analyzed using PCR. The aim of the present study was to obtain associations between data on distribution of different pathogens involved in PRDC in closed pig herds in Benelux using the TBS technique during different seasons and their relation with specific weather conditions.
Materials and Methods: 412 pig farms were sampled using the TBS technique over a 5-year period. In every herd, at least 30 coughing piglets were sampled in at least two age groups (3-5, 6-11 and 12-20 weeks of age). TBS were collected as described previously and analyzed using mPCR and/or dPCR (IVD GmbH, Germany). PCR results were reported as negative or positive for the presence of PRCV, SIV, PCMV, PCV2, A. pleuropneumoniae and H. parasuis. For PRRSV, strain type EU/US or both was also reported. Results were categorized and analyzed according to the season of sampling. Weather data were collected from a weather station localized in the center of the study area (Valkenswaard, NL).

Results: In piglets of 3-5 and 6-11 weeks of age, SIV, PRRSV-EU en Mhyo were the most prevalent pathogens. In fattening pigs, prevalences for Mhyo, PCV-2 and PRRSV-EU did not differ a lot among season. Evolution in the prevalences throughout the year during different seasons was clearly associated with humidity and temperature.
Conclusion: The present study clearly shows that different viral pathogens responsible for PRDC may be present during the post-weaning and fattening period. Following analysis of seasonal variation, it can be concluded that depending on the pathogen, a clear variation in seasonal impact in the PRDC is present. This is in accordance with recent observations for Mhyo in Spain. In conclusion, the present study showed that many respiratory pathogens are present during the post-weaning and fattening period, which may complicate the clinical picture of respiratory disease. Moreover, interactions between the PRDC pathogens and weather conditions could be shown in the present study over a period of 4 years.

Disclosure of Interest: None Declared

Keywords: PRDC, seasonality, TBS

Herd Health Management and Economy

O-HHM2-007

Identifying potential biomarkers to improve production in pigs

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Introduction: Work Package 5 of the Prohealth consortium aims to identify molecular markers that are associated with poor production in pigs. Microarrays are a powerful tool which will enable us to determine which genes in the affected tissues are up- or down-regulated as a result of the farm environmental condition. The application of software analysis packages such as GeneSpring will allow us to locate and pinpoint these genes to particular physiological or immunological pathways and help us to identify genes or sets of genes which have predictive value in identifying animals which are at risk.
Materials and Methods: As part of an EU wide consortium, Prohealth has access to samples from a variety of countries including Belgium, Spain, France and the Republic of Ireland. Agilent 4x44K microarrays will be used to analyse the changes in gene expression from animals reared in different commercial and experimental farms. This work focuses on data obtained from an experimental design performed at INRA (France) on Large-White pigs housed in poor or high hygiene conditions and exhibiting clear differences in growth performance and plasma immune status according to housing conditions. RNA from tissue samples preserved in RNAlater have been extracted, labelled and hybridised on the arrays at the University of Nottingham (UK). The arrays were analysed with a GenePix 4000B microarray scanner. Ingenuity Pathway Analysis will be used to recognise specific gene pathways and identify potential biomarkers for certain diseases and environmental conditions.
Results: Initial results from the microarrays have been compared with results obtained using a Biomark high-throughput RT-PCR device (Fluidigm) at INRA. Preliminary data indicates that the two methods show similar results. Initially, only the immune-related genes were analysed by microarray, since prior to microarray analysis, the Biomark analysis was used to assess expression of 90 immune-related genes in three different tissues from pigs bred in poor or high hygiene conditions. The array results indicated that over 30 immune-related genes were over-expressed in pigs reared on a high hygiene farm compared to a poor one.
Conclusion: Our initial results are promising and are comparable with other molecular methods such as the Biomark system. Many more genes other than the immune-related ones are expected to have differences in expression between pigs bred in two extreme hygiene conditions. Identifying the molecular pathways and the individual genes which are differentially expressed will allow us to define a set of biomarkers specific to particular diseases or environmental conditions. This work was conducted under the EU-funded PROHEALTH project.

Disclosure of Interest: None Declared
Keywords: biomarkers, Microarray

Oral Abstracts - Thursday 09 June 2016

Herd Health Management and Economy

O-HHM2-008

Case-control study to assess the importance of mycotoxins in tail necrosis in neonatal piglets

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Introduction: Tail necrosis in neonatal piglets was reported in several conventional pig herds in Belgium. The primary cause remains unclear. Mycotoxins are frequently mentioned to be involved, although a causal relationship has not been established yet. This case-control study aimed to investigate the involvement of feed-mycotoxins on the prevalence of tail necrosis in neonatal piglets.
Materials and Methods: Ten conventional pig herds with and ten without clinical signs of tail necrosis in neonatal piglets were visited between November 2014 and April 2015. Control herds were similar to case herds in terms of herd characteristics. No mycotoxin binders were present in the feed. One feed sample, provided to the sow at the time of sampling, was collected per farm. On each case herd, blood samples were taken from 5 sows with clinical signs amongst their offspring, and from 2 affected piglets (aged 1-3 days) per sow. Milk samples were also collected from these sows after injection of 2ml of oxytocine i.m.. A similar sampling scheme was used in the control herds. All samples were analyzed for mycotoxins and selected phase I metabolites via validated LC-MS/MS methods.
This study was approved by the ethical committee for animal experiments (EC 2014/123) and financed by Veepeiler-Varken.
Results: In total twelve different mycotoxins were detected in the feed and all feed samples contained at least one mycotoxin. DON was found in all feed samples: the average (± SD) concentration in case- and control herds was resp. 484 (± 212) µg/kg and 257 (± 89) µg/kg ($P<0.05$). All feed samples were below the EU threshold of 900 µg/kg DON. ZEN was found in 3 case and 2 control herds, with an average of resp. 156 (± 88) µg/kg and 108 (± 54) µg/kg. DON was detected in 89% of all plasma samples from sows, the average (± SD) concentration was 0.967 (± 0.692) ng/ml and 0.510 (± 0.311) ng/ml ($P<0.05$) for the case and control herds resp.. ZEN, -zearalenol and β-zearalenol were detected in a limited number of sow plasma samples. DON concentrations in piglet plasma samples were resp. 0.049 (± 0.051) ng/ml and 0.017 (± 0.015) ng/ml ($P>0.05$).
DON concentrations in feed were positively correlated with DON concentrations in sow plasma ($r = 0.70$), but weakly correlated with DON in piglet plasma ($r = 0.33$). DON concentrations in sow and piglet plasma were also weakly correlated ($r = 0.18$). Analysis of milk samples and of phase II metabolites, as biomarkers for DON and ZEN exposure is currently ongoing.
Conclusion: These results suggest a possible involvement of DON in the prevalence of neonatal tail necrosis, as there were significant differences in the concentration of DON in sow feed and sow plasma between case and control herds.

Disclosure of Interest: None Declared

Keywords: LC-MS/MS , Mycotoxins, Neonatal tail necrosis

Herd Health Management and Economy

O-HHM3-010

Association between biosecurity, productivity and antimicrobial use in Danish pig herds

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Introduction: Biosecurity is a key element of good farming practice and considered important to prevent disease spread within and between pig herds. Increased prevalence of disease in a pig herd usually results in decreased productivity and increased use of antimicrobials. One prevention strategy is to increase the focus on biosecurity. However, how effective is this?
This study aimed at elucidating the association between biosecurity, productivity measures and antimicrobial usage at farm-level. In total, 159 Danish conventional pig herds with sows and weaners (7-30 kilos) were included in the study.
Materials and Methods: For assessment of biosecurity on these farms, each farm owner or responsible operator was phone-interviewed in August-November 2015, using the questions from the online biosecurity scoring system Biocheck.ugent®. Biocheck provided assessment of both internal and external biosecurity, using pre-weighted questions about the practices and procedures in a farm, divided into different sub-categories. Productivity data (e.g. average daily gain, feed conversion ratio, and mortality) from 2014 from each of these herds were kindly provided by SEGES in Denmark. In line, the total antimicrobial prescription in the same year was extracted from the Danish VetStat Database, and used as a measure of the antimicrobial usage, given in Animal Daily Doses (ADD).
The association between the productivity and antimicrobial usage outcomes and the biosecurity scores from Biocheck will be evaluated using linear regression models including potential confounders such as herd size and herd health status.
Results: The assessment of the biosecurity in Danish pig herds revealed that the average level of external biosecurity was higher (86% out of max 100%) than the internal biosecurity (67% out of max 100%). This is probably a result of the Danish SPF (Specific Pathogen Free) system; a health and production system with high focus on external biosecurity. Today, 78% of Danish sow herds are enrolled in the SPF system and the experience is that the remaining herds are also following many of these rules regarding for example purchase of animals, transportation, pest control and supply of water and feed. Furthermore, the level of external and internal biosecurity was higher in Denmark compared to other EU countries that have also been using Biocheck.
Conclusion: The results from the on-going statistical modeling will show to which extent the biosecurity measures were associated with the level of antimicrobial use and productivity in herds.
All results from this study will be ready for presentation at the IPVS 2016.

Disclosure of Interest: None Declared

Keywords: antimicrobial usage, biosecurity, production parameters

Herd Health Management and Economy

O-HHM3-011

Early warning of diarrhea and pen fouling in growing pigs using sensor-based monitoring

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Introduction: Today, pig farmers typically asses the health of their pigs while walking through their herd as part of the daily routine. In modern pig production, however, a herd will consists of thousands of pigs in a few hundred pens. If a farmer were to spend just two minutes observing each pen to assess the overall health of the pigs, this alone would easily take 4-5 hours. As real farmers won't take this time, problems can easily be overlooked, leading to needless welfare problems and costs. Implementing existing sensor technology enables automatic monitoring 24/7, and detection algorithms can then identify specific pens in need of extra attention. Here we evaluate the value of monitoring live weight, feed usage, humidity, drinking behavior and pen temperature in relation to early warnings of diarrhea and pen fouling in slaughter pigs.
Materials and Methods: We used data collected in 16 pens (8 double-pens) between November 2013 and December 2014 at a commercial Danish farm. During this time, three new batches were inserted. We monitored the mean live weight of the pigs per pen (weekly, only in 4 pens), feed usage per double-pen (daily), humidity per section (daily), temperature at two positions per pen (hourly), water flow per double-pen (liters/hour/pig) and drinking frequency per pen (activations/hour/pig). Staff registrations of diarrhea and pen fouling were the events of interest. The data were divided into a learning set (15 events) and a test set (18 events).
The data were modeled on pen level with a multivariate dynamic linear model, which was built using the learning set. Alarms were raised if the unified forecast errors were above a control limit for a sufficient number of consecutive hours (0-25) during a 24 h day. An alarm up to 3 days before or 1 day after an event observation was considered a true positive. If no alarm was raised within this window, it was considered a false negative. True negatives and false positives were counted per day. The predictive values of the various variables were estimated based on how much their omission affected the performance, measured as the area under the receiver operating characteristics curve (AUC).
Results: We achieved an AUC of 0.83 when all variables were included. Omitting live weight and humidity had no effect. Omitting drinking behavior, temperature, and feed usage reduced the AUC to 0.70, 0.81, and 0.82 respectively.
Conclusion: We show that drinking behavior, pen level temperature, and to a lesser extend feed usage, all hold information which is applicable for indiscriminate early warnings of pen fouling and diarrhea. We could not show any added value from including live weight or feed usage.

Disclosure of Interest: None Declared

Keywords: Dynamic linear model, early warning, monitoring

Herd Health Management and Economy

O-HHM3-012

Effect of trimming long toes of sows on longevity, productivity, and economic return

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Introduction: Trimming long toes has become a topic of interest for improving sow retention and herd productivity. However, there is limited scientific evidence that trimming long toes in commercial sow farms results in improved productivity and therefore is economically justified. Therefore, the objective was to evaluate the effects of trimming long toes, overgrown heels and long dewclaws on sows and the impact this has on longevity and productivity to determine economic return.
Materials and Methods: Previous published literature suggests that average toe length for sows is 55 mm from coronary band to tip of toe. Therefore, seven hundred and sixty parity 2 and 3 sows with toes longer than 60 mm at mid-gestation either had toes trimmed or left untrimmed. Toes were evaluated for toe length, long and cracked dew claws, heel erosion or overgrowth, and heel and wall cracks. Time to trim toes was measured. All sows had the opportunity to have 3 farrowing events and standard litter traits were recorded for each farrowing event as well as total productivity. If a sow was removed from the herd due to being culled, mortality, or euthanized, the date and reason was recorded. Data were analyzed using Proc GLM in SPSS and Proc GLIMMIX in SAS with the first farrowing litter data as a covariate. Sow was the experimental unit.
Results: Parity 2 and 3 sows that were determined to have toes longer than 60 mm had rear toe lengths averaging 78 mm, while 97% of those sows had issues with dew claws and 83% of those sows had heal sole cracks. Trimming toes takes as long as 31 minutes or as quick as 3 minutes, with an average of 8 minutes per sow. Trimming long toes increased the average number born alive ($P < 0.05$) of sows that farrowed for the third farrowing by 0.6 piglets than sows with long toes that were left untrimmed. However, trimming long toes did not improve ($P > 0.10$) any litter traits for the 2nd farrowing or sow removal compared with sows that were left untrimmed. Six percent of sows in the herd had toes longer than 60 mm.
Conclusion: Trimming long toes of parity 2 and 3 sows resulted in 0.6 more pigs born alive during the third farrowing event after trimming, but no improvement in sow retention or total sow productivity occurred. The return over expense (ROE) for trimming the 6% of sows with long toes was calculated with capital and labor cost for year one and labor cost for year 2 divided by the value of increased pigs born alive which occurred in year 2. First year ROE is 0:1 and year two ROE is 2:1. Due to a low ROE for trimming toes that were longer than 60 mm of parity 2 and 3 sows, it is suggested to focus on prevention of long toe growth.

Disclosure of Interest: None Declared

Keywords: long toes, trim, productivity

Oral Abstracts - Thursday 09 June 2016

Herd Health Management and Economy

O-HHM3-013

Physical castration affects the health and productive performance of pigs in the suckling period

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Introduction: European agreements specify that from 2012 physical castration of pigs should be performed with prolonged analgesia and/or anesthesia and that it should be abandoned totally by 2018. Nowadays, physical castration is still practiced in most EU countries to avoid undesirable sexual or aggressive behavior and to avoid boar taint. However, available evidence shows that castration is painful and may have a detrimental influence on health. This study investigated the clinical and productive impacts of physical castration in the suckling period with a large enough group of animals to reach statistically relevant conclusions.
Materials and Methods: A total of 3696 male pigs 3 to 6 days old from 721 litters of two different farms were included in the study. Within each litter, half of the males were kept as entire males (EM) and half were physically castrated (CM). Physical castration was conducted by a trained farmer, using a non-steroidal anti-inflammatory drug before castration. Average daily gain (ADG), body weight at weaning (BWW), percentage of pre-weaning mortality (PWM) and antibiotic usage were measured. Productive performance data were analyzed using a linear mixed model with the fixed effect of treatment, block effect of litter and random effect of farm, enrolment batch, room, and litter within enrolment batch. Mortality and percentage of pigs treated with antibiotics were analyzed as binary variables using the chi-squared test. The effects were also analyzed in the 25% lightest and 25% heaviest pigs at birth.
Results: No differences in BWW and ADG were observed between treatments. However, PWM was higher in CM than in EM (6.3% vs 3.6%; $P<0.001$), mainly due to complications following castration (0.9% vs 0; $P<0.001$), meningitis (1.2% vs 0.6%; $P<0.05$) and runt pigs (1.3% vs 0.5%; $P<0.01$). The difference in PWM was higher in the light group (12.7% vs 6.6%; $P=0.002$), even though ADG and BWW were not affected this group. 1.5% of light CM pigs died at castration. In the heaviest pigs group PWM was not affected by castration and no CM pigs died at castration, but EM tended to show higher ADG ($P=0.06$) and showed higher BWW (8.0 kg vs 7.8 kg; $P<0.05$) than CM. Percentage of pigs treated with antibiotics was not affected by castration, a result likely influenced by differences in PWM.
Conclusion: Physical castration promotes productive losses in the suckling period because it causes an increase in PWM, especially in pigs born with a lower body weight, and may affect ADG and BWW in pigs born with a heavier body weight. Main causes of mortality related to physical castration were the procedure of castration, meningitis and runt pigs.

Disclosure of Interest: None Declared

Keywords: Physical castration, Suckling piglets

CSF/ASF

O-VVD4-005

Detection Of African Swine Fever Virus In Blood And Tissue Exudates Of Wild Boar And Swine By Cross-Priming Amplification With Lateral Flow-Dipstick.

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Introduction: African Swine Fever (ASF) is an infectious viral disease of swine and wild boar. At Poland, African Swine Fever virus (ASFV) was first detected in February 2014. Up to now 76 cases in wild boar and 3 outbreaks in swine were confirmed by real-time PCR with universal probe library (UPL), enzyme-linked immunosorbent assay (ELISA) and immunoperoxidase test (IPT). In spite of high reliability of these techniques a new simple and portable methods are required. Recently, isothermal amplification assays showed to be efficient for rapid detection of a number of swine pathogens including ASFV. Therefore, cross-priming amplification method (CPA) with lateral flow dipstick (CPA-LFD) was developed and applied within this study for ASFV detection in blood and tissue exudates from wild boar and swine.
Materials and Methods: Blood and tissue exudates originated from totally 76 cases and 3 outbreaks which occurred between 2014 and 2015. About 10 µl of each blood or tissue exudate was diluted in 90 µl of APO buffer (Novazym, Poznan, Poland) and incubated for 10 min. The CPA primers were designed to target conservative fragment of ASFV p72 gene. The CPA temperature was optimised in a water bath in range from 55.4°C to 66.9°C and in time from 30 to 120 min. The assay volume was 15 µl, and contained: 7.5 µl of Isothermal Mastermix (OptiGene, Horsham, West Sussex, United Kingdom) and different concentration of 5 CPA primers. The inner primers (2a and 3a) were labelled with biotin and FITC, respectively. Lateral flow dipstick analysis was conducted by hybridization step followed by submerging of LFD strip (Milenia Biotec, Germany) in the mixture to visualize positive result as the presence of two lines and negative as the single control line. The reference UPL real-time PCR method was applied to compare CPA-LFD obtained results.
Results: The conducted CPA optimisation showed the most efficient amplification underwent at 56.2°C starting from the 45 min. The CPA detection limit was 10^{-6} dilution of DNA extracted from the standard ASFV strain and was equal with the reference UPL real-time PCR method. The CPA successfully detected the presence of ASFV DNA in diluted blood and all tissue exudates from 76 cases in wild boar and 3 outbreaks in swine without DNA extraction.
Conclusion: The effective and reliable detection of ASFV has an important epidemiological and economical aspect. The CPA was highly sensitive and may be conducted in a water bath without application of thermocyclers. The developed CPA was capable to specifically detect ASFV DNA in the blood and tissue exudates directly collected from the infected wild boar and swine.
Funded by KNOW (Leading National Research Centre - Leading Laboratory)
Disclosure of Interest: None Declared
Keywords: African swine virus, cross-priming amplification, detection

CSF/ASF

O-VVD4-003

Classical swine fever virus (CSFV) detection using oral fluid samples from CSFV-inoculated pigs

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Introduction: Re-emergence of CSFV in free areas is a continual risk and the ability of CSFV to spread over long distances to areas free of CSFV is well-documented (1). Accurate and rapid surveillance/testing procedures are mandatory for detection, control, and/or elimination because (1) CSFV infections cannot be differentiated from endemic pathogens, e.g., PRRSV, salmonellosis, on the basis of clinical signs and because (2) CSFV can be shed throughout a pig's lifetime without overt clinical signs. The purpose of this study was to evaluate the detection CSFV by RT-PCR using oral fluid samples.
Materials and Methods: 30 unvaccinated, CSFV antibody-negative pigs (35-40 kg) were individually housed from day post inoculation (DPI) -14 to 28 in research facilities at Chulalongkorn University (Bangkok, Thailand). At DPI 0, pigs were IN inoculated with CSFV (ALD strain, $1x10^5$ $TCID_{50}$/ml). Individual oral fluid samples were collected daily from DPI -14 to 28. For comparative purposes, serum samples were collected at DPI -14, -7, 0-, 1, 2, 3, 4, 5, 6, 7, 10, 14, 17, 21, and 28. Oral fluid (n = 504) and serum (n = 276) samples were tested for CSFV RNA using a commercial kit (LSI VetMax™ CSFV (PPC), Life Technologies, Le Bois Dieu, Lissieu, France).
Results: Oral fluid volume collected on a daily basis was variable from 1-50 ml/pig/time. No false positive oral fluid CSFV rRT-PCR results were observed, i.e., 100% specificity was observed among samples collected prior to inoculation (n = 90). CSFV RNA was detected in oral fluid samples from DPI 4 (2/29 pigs) through DPI 28 (5/15 pigs). The highest proportion of positive results was observed on DPI 12 (12/12 pigs). An analysis of serum and oral fluid RT-PCR results (n = 147) showed a statistically significant association (p = 0.0001).
Conclusion: CSFV RNA was detected in oral fluid specimens collected from individual CSFV-inoculated pigs before the appearance of clinical signs, during the course of clinical signs, and after recovery. Thus, the data suggest that CSFV surveillance could be done using oral fluid samples. This approach could be particularly useful in for the detection of inapparent CSFV infections.
Disclosure of Interest: None Declared
Keywords: classical swine fever virus, Oral fluids, RT-PCR

Oral Abstracts - Thursday 09 June 2016

PCV2

O-VVD4-002

Persistent experimental PCV2a infection and development of porcine dermatitis and nephropathy syndrome without evidence of seroconversion

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Introduction: In growing pigs, porcine circovirus type 2 (PCV2) has been identified as an important factor in respiratory and enteric disease and PCV2 is also capable of inducing systemic disease. An association of PCV2 with porcine dermatitis and nephropathy syndrome (PDNS), while not confirmed under experimental conditions, is also suspected. The objective of this study was to determine long term infection dynamics of PCV2 and PPV1 in high health status pigs and produce a stock of anti-PPV1 and anti-PCV2a serum.
Materials and Methods: Six caesarian-derived colostrum-deprived pigs were randomly divided into two groups and rooms of three pigs and were experimentally infected with PCV2a or PPV at 5 weeks of age. Blood was collected every 3 days for the first 21 days and then weekly thereafter. Serum samples were tested for presence and amount of DNA by PCV2 real-time PCR and PPV real-time PCR. Serum samples were also tested for antibodies by an in-house and a commercial PCV2 ELISA, a PPV HI assay and a commercial PPV ELISA. At necropsy, tissues were collected for histopathology and PCV2 IHC to determine tissue PCV2 levels.
Results: All PCV2 infected pigs were PCV2 viremic at day 3, remained viremic for the duration of the study, and developed severe clinical illness. Pig 124 developed systemic PCVAD (necropsy on day 28), pig 121 developed PDNS (necropsy on day 56) and pig 125 developed severe chronic PCVAD including fibrosing hepatitis and enteritis (necropsy on day 106). Microscopically all three pigs had severe lymphoid lesions consistent with PCVAD with high levels of PCV2 antigen. Only one of three pigs developed detectable anti-PCV2 antibodies starting at day 48; however, the levels remained low through day 106 which was confirmed by two independent ELISAs. The age- and source-matched pigs infected with PPV remained clinically healthy, seroconverted to PPV1 by day 12 and developed high levels of anti-PPV antibodies soon thereafter and persisting through termination of the study at day 42. None of the PPV1 pigs had any lesions at necropsy.
Conclusion: Under the study conditions, we contrasted the differences in responses of pigs to two distinct small single-stranded DNA viruses in CDCD pigs. We experimentally reproduced systemic PCVAD and PDNS in PCV2a-infected pigs. Our group has infected hundreds of pigs with various PCV2 isolates (PCV2a, PCV2b, PCV2d) over the last 10+ years and this is the first time we have been able to reproduce classic PDNS lesions. This study further highlights the unique immune response to PCV2a in some pigs and the importance of controlling PCV2a in herds with PCVAD and PDNS.

Disclosure of Interest: None Declared

Keywords: Experimental Infection, Porcine Circovirus 2, Porcine dermatitis and nephropathy syndrome

PCV2

O-VVD4-004

Vaccination can select vaccine-escape mutants in PCV2

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Introduction: Highly efficient commercial vaccines induce protective immunity against PCV2. However, the virus that holds a high mutation rate is still circulating in vaccinated farms. Thus, and because of new mutant strains and observations of PCVD in vaccinated herds, there is growing concern about PCV2 strains capable to escape vaccination. Different authors suppose recombinations or mutations within ORF2 as a possible reason and demand more research to evaluate the effect of vaccine pressure in PCV2 and the potential emergence of vaccine-escape mutants.
Materials and Methods: Based on 2156 samples from individual pigs of 315 herds we describe a high effectivity of vaccination between 2008 and the third quarter of 2011. In this period virus load dropped continuously, until the threshold of quantification was deceeded. Between 2011 and 2014 however, virus loads re-increased, some reaching concerning thresholds, although most of the herds were still vaccinated. 62 randomly selected samples from vaccinated (n = 28) and non-vaccinated (n = 26) herds were completely sequenced. Polymorphisms were analysed for associations to vaccination status, major genotype (PCV2a/PCV2b), and virus load.
Results: PCV2a genotypes were decreased by vaccination from 24% to 3.6%, while the PCV2b genotypes increased. One SNP at nucleotide 1182 (g.1182G>T), involved in capsid epitope formation, (amino acid 185 [p.185L>M]) was significantly associated with vaccination and viral load, independent from the PCV2 genotype (2a/2b). Vaccination was found to drive out the methionine variant, in favour of the leucine variant.
Conclusion: These results provide evidence for a selectional impact of vaccination on the PCV2 sequence, especially on nucleotides involved in epitope formation. Such variation might be responsible for the observed re-increase of PCV2-loads in samples, starting from the end of 2011 in our samples. Capability of PCV2 to escape vaccination does not seem to be a question of genotypes (2a, b or others), but of distinct epitope variation that can occur independently from these major genotypes. Our results prove a role of amino acid 185, but theoretically amino acid substitution at other positions involved in antibody binding should lead to similar consequences. The focus on major genotypes (2a, b…) that are defined by a multitude of mutations is extremely forwarding for evolutionary or epidemiological issues, but can make it harder to detect effects of single mutations, especially if variants segregate within these major genotypes. This aspect needs more consideration in future studies dealing with the sequence of PCV2.

Disclosure of Interest: None Declared

Keywords: PCV2, vaccine escape

PCV2

O-VVD4-001

Analysis of PCV2-neutralizing antibodies and neutralizing epitope(s)

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Introduction: Vaccination is the most efficacious procedure to curtail Porcine circovirus 2 (PCV2)-associated diseases (PCVAD). However, it is well known that while commercial PCV2 vaccines protect pigs from clinical signs, they do not prevent infection. Experimental studies indicate that PCV2 vaccine-induced virus-neutralizing antibodies play a major role in protection from PCVAD. However, the immune response to PCV2 vaccination of pigs in a farm environment is less clear. This study enlightens the role and molecular targets of neutralizing and non-neutralizing anti-PCV2 antibodies of pigs from modern pig production facilities.
Materials and Methods: We examined 160 pig sera from 13 pig farms in Canada (80 animals vaccinated with commercial PCV2 vaccines following the instructions of the manufacturers; 80 animals not vaccinated against PCV2). We determined levels of PCV2 neutralizing and non-neutralizing anti-PCV2 antibodies and the copy number of PCV2 genomes present in sera from these farmed pigs. We established a particle-based ELISA and Western Blot system representing conformational and linear epitopes, respectively. Using these methods, we assessed sera from selected pigs to differentiate reactivity against conformational and linear epitopes, and correlated these data with the neutralizing properties of the sera.
Results: 1. Serum levels of PCV2 genome copies were not different between vaccinated and non-vaccinated animals. 2. In the high-risk group for disease (nursery/weaner pigs), vaccinees had significantly higher PCV2-neutralizing antibodies than their non-vaccinated counterparts. In all three other age groups, we observed no significant differences in PCV2-neutralizing antibody levels between vaccinated and non-vaccinated pigs. 3. Most of the older animals had substantially higher titres of PCV2 neutralizing antibodies than younger pigs, regardless of being vaccinated or not. 4. Neutralizing antibodies targeted conformational but not linear epitopes. 5. PCV2-neutralizing epitopes were present of the surface of virus particles.
Conclusion: Vaccination has a positive impact on PCV2-neutralizing antibody induction in the group of pigs with the highest risk for developing PCVAD. However, titres of vaccine-induced PCV2 neutralizing antibodies are low, and are thus likely not sufficient to prevent infection with PCV2. Natural exposure to PCV2 seems to induce a much higher neutralizing antibody response than vaccination. Current PCV2 vaccines might not provide sufficient amounts of PCV2 neutralizing epitopes to pigs to quickly induce high neutralizing antibody titres. Neutralizing epitopes appear to be present on the surface of intact virions. This could be a focus of future PCV2 vaccine developments.

Disclosure of Interest: None Declared

Keywords: farmed pigs, Neutralizing antibodies, Neutralizing epitope

SIV

O-VVD5-016

Reassortment between swine H3N2 and 2009 pandemic H1N1 generated diverse genetic constellations in influenza viruses circulating in United States pigs

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Introduction: Influenza A virus (IAV) is a significant pathogen to the swine industry. Since its introduction in 2009, the H1N1 pandemic virus (H1N1pdm09) has been repeatedly transmitted from humans to swine, but onward transmission in U.S. swine was mostly restricted to its internal genes. Reassortment between the H1N1pdm09 and endemic swine viruses resulted in substantial evolution of IAV and led to the circulation of different genomic constellations in pigs. We conducted phylogenetic analyses of H3N2 IAV circulating in swine from 2009 to 2014 in the U.S. and compared the pathogenesis and transmission of six representative genotypes in pigs.
Materials and Methods: Whole genome phylogenetic analyses were performed on 496 H3N2 IAV isolated from U.S. swine from 2009 to 2014. Isolates were classified into genotypes when the eight gene segments phylogenies resulted in a unique genome constellation. Six swine H3N2 genotypes were selected to be tested *in vivo*: two with HA of cluster IV-A, two IV-B, and two IV-F with varied internal gene constellations. Pigs were challenged with each assigned virus and indirect contact pigs were placed at 2 days post infection (dpi) to evaluate transmission. Nasal swabs were collected from primary and indirect contact pigs. At 5 dpi, macroscopic and microscopic lung lesions were evaluated in challenged pigs, and bronchoalveolar lavage fluid was collected.
Results: At least 54 different swine H3N2 genotypes were identified in the U.S. The most common genotype (32.2%) started being identified in 2011, and contains a cluster IV-A HA gene, 2002-lineage NA gene, H1N1pdm09 M gene, and remaining genes of triple reassortant internal gene (TRIG) origin. After 2011, there was a rapid increase in predominance of the H1N1pdm09 M gene in all genome constellations. More than 65% of the isolates had at least 1 H1N1pdm09 internal gene. Although the six genotypes we tested efficiently infected pigs producing similar lung viral titers, they resulted in different degrees of pathology (mild to moderate lung lesions). Nasal viral shedding also differed among viruses, but all were transmitted to indirect contacts. Differences in pathology, lung replication and viral shedding did not seem to correlate with individual genes.
Conclusion: These results highlight the great diversity of H3N2 genotypes circulating in U.S. swine after 2009, and 6 common genotypes were shown to be fully virulent and transmissible in swine. This continued evolution could have important implications to the control of this disease by the swine industry and to the risk of zoonotic infection in humans due to incorporation of the H1N1pdm09 genes.

Disclosure of Interest: None Declared

Keywords: Genotypes, Influenza A Virus, swine H3N2

Oral Abstracts - Thursday 09 June 2016

SIV

O-VVD5-015

Could avian H9N2 influenza viruses become a threat to swine? Lessons from experimental studies in the pig.

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Introduction: It is a classical but unproven hypothesis that pigs can serve as intermediate hosts between birds and humans in the generation of novel pandemic influenza viruses. Yet the single pandemic virus of likely swine origin is the 2009 pandemic H1N1 virus (2009 pdm), a virus with genetic components of swine, human and avian origin (reassortant). The latter virus has become well adapted to pigs and humans and is now widespread in both species. Avian H9N2 viruses are endemic in Eurasian poultry and they are considered potential pandemic candidates because they cause sporadic dead-end infections in pigs and humans. However, H9N2 viruses are not adapted to mammals since they lack capacity to spread within the human or swine population. We and other researchers have previously examined whether H9N2 viruses could become adapted to pigs by two strategies: serial pig passages which are known to force viruses to mutate; and reassorment replacing H9N2 internal genes by those of a swine adapted influenza virus (SIV), the 2009 pdm. Both approaches slightly enhanced virus replication and transmission, but transmission was still inefficient when compared with SIV. Thus, we aimed to examine whether such reassortant H9N2 virus could become better adapted to swine after serial pig passages.
Materials and Methods: We performed four pig transmission experiments with four different viruses: a non-passaged reassortant virus containing A/quail/Hong Kong/G1/97 (H9N2) surface genes and A/California/04/09 (2009 pdm) internal genes; another reassortant virus with the same genetic constellation but passaged seven times in pigs and both parental viruses, the avian H9N2 and the 2009 pdm SIV. In each experiment 3 animals were individually housed and intranasally inoculated with the selected virus. Two days later, 2 direct contact animals were co-housed with each inoculated pig. Nasal swabs were collected daily from all pigs for virus titration.
Results: All inoculated pigs excreted medium to high amounts of virus during at least 5 days. The 2009 pdm was the single virus for which all 6 contact pigs shed high amounts of virus during 5 days. As expected, the parental H9N2 and the non-passaged reassortant H9N2 viruses were not efficiently shed by any contact pig. In contrast, the pig-passaged reassortant H9N2 virus was shed in high amounts by 4 of the 6 contact pigs.
Conclusion: Our data suggest that serial passages induced mutations in the H9N2 reassortant virus, which improved its replication and transmission. Genetic analysis of this virus is still pending. We demonstrated that adaptation of avian H9N2 viruses to pigs is a complex multi-step process. H9N2 could pose a threat for pigs and humans if this process would occur in nature.

Disclosure of Interest: None Declared

Keywords: 2009 pandemic H1N1, H9N2 avian influenza virus, Transmission

SIV

O-VVD5-014

Effect of sow vaccination on the detection of influenza A virus in pigs at weaning

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Introduction: Most common influenza A virus (IAV) control measures include sow vaccination using prefarrow, mass or a combination of prefarrow and mass vaccination protocols with either commercial, autogenous or both vaccines. Piglets prior to wean are important in the maintenance of influenza infections in breeding herds as well as in the dissemination of the virus into wean-to-finish facilities. The aim of this study was to evaluate the effect of sow vaccination protocol and type of vaccine used on the detection of IAV in groups of pigs at weaning.
Materials and Methods: There were 52 farms enrolled which were located in 6 U.S. states. Thirty nasal swabs were collected monthly for 6 months in each farm from pigs prior to wean. Sampling timeframe was between January and November 2013. One piglet was conveniently selected from each of 30 litters and nasal swabs collected. Swabs were tested in pools of 3 to detect influenza matrix gene by RT-PCR at the UMN-VDL.
Generalized linear mixed logistic (GLMM) regression models were used with influenza RT-PCR results (positive/negative) at group level as the outcome, and vaccination protocol and type of vaccine as fixed predictors within univariable models. Farm and season (time) were incorporated as random predictors.
Results: Sixty three percent of herds vaccinated against IAV. Out of the 33 herds that vaccinated, 45%, 36% and 19% had prefarrow, mass and prefarrow and mass vaccination protocols, respectively. Out of 32 vaccinated farms, 53%, 31% and 16% used commercial, autogenous or a combination of commercial and autogenous vaccines, respectively. One production system did not report the type of vaccine used and was excluded from the analysis. Overall, 48% of the herds tested positive and of these, 44% of vaccinated and 58% of non-vaccinated herds tested positive at least once. Moreover, 25% of groups of pigs at weaning tested positive with vaccinated herds having 16% of positive groups compared to 40% of the non-vaccinated herds.
The odds of detecting positive groups significantly decreased by 84% in vaccinated herds. Prefarrow and mass vaccination protocols significantly decreased the odds of positive groups compared to no vaccination. There were no significant differences between prefarrow and mass vaccination protocols. Commercial vaccines significantly decreased the odds of having positive groups of pigs at weaning compared to no vaccination, and a similar trend was observed for autogenous vaccines. There were no significant differences between commercial and autogenous vaccines.
Conclusion: Overall, our results indicated that IAV sow vaccination may be an effective tool at reducing the prevalence of IAV in piglets at weaning.

Disclosure of Interest: None Declared
Keywords: Influenza A Virus, sow vaccination, weaning pigs

SIV

O-VVD5-017

Current trends from the USDA influenza a virus in swine surveillance system

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Introduction: A U.S. national surveillance system for influenza A viruses (IAV) in swine was initiated in 2009 with increasing participation to the present day. The objectives are to monitor genetic evolution of IAV in swine, make isolates available for research, diagnostic reagents, and vaccine development through an IAV repository, and provide publically available sequence data for animal and human health purposes. These data can be used to investigate patterns of virus evolution or movement, regional differences, and temporal changes. We report analyses of IAV isolated from fiscal years 2014-15 (FY14-15) since earlier data were reported previously.
Materials and Methods: Routine samples submitted for diagnostic investigation of respiratory disease are included in the USDA system through the National Animal Health Laboratory Network. Testing includes RT-PCR screening for IAV, subtyping RT-PCR, virus isolation, and sequencing of the HA, NA, and M genes. Whole genome sequencing is conducted on a subset of viruses each month. Sequences are deposited into GenBank and phylogenetic analyses of available HA, NA, and M genes from viruses isolated in FY14-15 were performed.
Results: Approximately 15,000 accessions were submitted during FY14-15 and ~1600 viruses isolated with sequences available in GenBank. Phylogenetic analyses revealed changes in dynamics among multiple clades of H1N1, H3N2, and H1N2 co-circulating in U.S. swine. Sequences were categorized into clades based on the HA genes: H1α, H1β, H1γ, H1δ1, H1δ2, H1pdm09, H3 IVA-F or human seasonal H3; and NA genes: classical N1, H1pdm09 N1, 1998-N2, 2002-N2, or human seasonal N2. The predominant HA and NA clade combinations during this period were H1γ/classical N1, H3 IV-A/2002-N2, H1δ1/2002-N2. However, the major genotypes did not replace the minor genotypes that continued to persist at low or sporadic levels. The H1N1pdm09 M gene was exclusively detected in all genotypes. Notable was the continued detection of an emerging IAV with a contemporary human seasonal H3 and swine classical N1 or 2002-N2. Some differences between regions were observed which suggest that regional decisions on vaccine components could be considered.
Conclusion: Surveillance and reporting of IAV in U.S. swine has increased dramatically since 2009, and we demonstrated continued genetic diversity, regional trends, and detection of emerging lineages in FY14-15. These results can inform vaccine strain selection and potential need for diagnostic updates. Furthermore, monitoring viral temporal and spatial patterns may indicate a need for changes in production practices that facilitate viral migration between and within U.S. states and regions.

Disclosure of Interest: None Declared

Keywords: Evolution, Influenza A Virus, surveillence

Oral Abstracts - Friday 10 June 2016

O-IV-001

Pathogenesis comparison and cross-protection efficacy of the U.S. PEDV prototype and S-INDEL-variant strains in weaned pigs

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Introduction: Two strains (U.S. prototype [P] and S-INDEL-variant [V]) of PEDV are currently circulating in the U.S. Pathogenesis comparison of the two strains in 5-day-old pigs showed that the V-strain was less virulent than the P-strain. However, PEDV pathogenicity is age dependent. Also, understanding the cross-protection between two strains is imperative for PEDV vaccine development. In the current study, the pathogenesis difference and the cross-protection efficacy between the two U.S. PEDV strains were evaluated in weaned pigs.
Materials and Methods: 85 PEDV-naïve 3-week-old (3w) pigs were divided into 7 groups. Pigs were orogastrically inoculated with negative media (N), a P-strain isolate (P), or a V-strain isolate (V) at Day 0 (D0) followed by challenge at D28, with 10^5 $TCID_{50}$/pig at each point. Seven groups were designated according to 1st/2nd inoculation: P/V (15 pigs), V/V (15 pigs), N/V (15 pigs), P/P (10 pigs), V/P (10 pigs), N/P (10 pigs), N/N (10 pigs). 5 pigs from the P/V, V/V, and N/V groups were necropsied at D4 and 5 pigs from all groups were necropsied at D34 to evaluate gross and microscopic lesions. The remaining 5 pigs/group were kept until D56 to evaluate virus shedding and antibody (Ab) responses by indirect fluorescent antibody (IFA) assay and virus neutralization (VN) test.
Results: P-strain-inoculated 3w pigs shed more viruses in feces, and had more severe intestinal lesions at D4, than V-strain-inoculated 3w pigs. Interestingly, after the 2nd challenge at D28 (pigs 7w), the N/V group shed more viruses than the N/P group and had more severe lesions at D34 than the N/P group.
After the 1st inoculation, the P/P, P/V, V/V and V/P pigs started to develop PEDV Ab from D7-14; Ab titers increased slightly after the 2nd challenge and were maintained through D56. The N/P and N/V groups were PEDV Ab negative until D42 when Ab became detectable and were maintained through D56. The N/N group remained virus and Ab negative throughout the study. After the 2nd challenge, fecal viral shedding in the P/P, V/P, V/V, and P/V groups was all significantly lower than the N/V and N/P groups. Both P/V and V/V groups had fewer lesions than the N/V group at D34. Due to mild lesions in the N/P group at D34, distinct lesion differences among the N/P, V/P and P/P groups were not observed.
Conclusion: 1. P-strain was more virulent than V-strain in 3w pigs but opposite in 7w pigs under the conditions of this study.
2. P-strain immunization provided protection against P-strain challenge and cross protection against V-strain challenge.
3. V-strain immunization provided protection against V-strain challenge and partial cross protection against P-strain challenge.

Disclosure of Interest: None Declared

Keywords: Cross-protection, Pathogenesis, weaned pigs

O-IV-002

PRRSV MLV vaccination in the presence of maternally-derived neutralizing antibody

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Introduction: Lopez et al. (2007, Clin Vaccine Immunol 14:269-275) reported that PRRSV neutralizing serum antibody in 15-day-old piglets did not stop PRRSV infection, replication, and transmission, but did reduce viremia and virus shedding. The goal of this study was to further explore the observations made by Lopez et al. (2007). Specifically, our objective was to quantify the effect of maternally-derived neutralizing antibody on post-vaccination viremia in piglets inoculated with PRRSV MLVs.
Materials and Methods: The study was performed in a PRRS stable breeding herd that had been vaccinated with Ingelvac® PRRS MLV 6 months earlier. To conduct the study, serum samples were collected from piglets 2-3 days post-farrow and tested for serum neutralizing (SN) antibody against each MLV vaccine. 60 piglets representing a range of SN antibody titers were selected from 10 sows (P1 to P8) and equally allocated into two vaccine treatment groups (A, B), placed in two different rooms, and vaccinated at three weeks of age. Group A (n = 30) received a full dose of Ingelvac PRRS® MLV and Group B (n = 30) received a full dose of Fostera® PRRS.
Results: One week post vaccination, serum samples were tested by PRRS qRT-PCR, with the following results:
Viremia post-vaccination (qRT-PCR)

Log2 SN Titer	Pigs	Pos	Neg	% Viremic
2	1:4	5	5	0 100%
3	1:8	9	6	2 67%
4	1:16	6	4	2 67%
5	1:32	2	2	0 100%
6	1:64	6	2	4 33%
7	1:128	6	1	5 17%
8	1:256	10	0	10 0%
9	1:512	16	3	13 19%

A regression analysis between the proportion of PRRSV qRT-PCR-positive pigs post vaccination and vaccine-specific neutralizing antibody titers (log2) at 2-3 days of age showed that the two variables were strongly associated ($p \leq 0.05$, R-squared = 71.5%), with a negative correlation (r = -0.85). That is, higher SN titers were associated with the absence of viremia.
Conclusion: These results supported the observations of Lopez et al. (2007). That is, SN antibody titers and PRRS qRT-PCR viremia post-vaccination inversely associated. Importantly, the results imply that shedding and transmission of vaccine virus could be reduced by intentionally enhancing the levels of maternally-derived neutralizing antibody in piglets prior to vaccination. Additional research is required to confirm this effect and to evaluate the effect of this approach on the onset and level of modified live vaccines-induced immunity.
Disclosure of Interest: None Declared
Keywords: PRRS MLV vaccine, PRRSV neutralizing serum antibody

O-IV-003

Combined PCV2 and M. hyopneumoniae piglet vaccination has a positive impact compared with Mhyo vaccination only, in a subclinically PCV2 infected farm

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Introduction: Besides PMWS (Post Weaning Multi-systemic Syndrome) or PCVD-systemic disease, PCV2 is also involved in many subclinical infections. In addition, PCV2 and *Mycoplasma hyopneumoniae* (Mhyo) infection results in a negative effect on performance. PCV2 and Mhyo vaccinations are an efficient tool to reduce mortality, lesions, viremia and to improve growth. **In this controlled and randomised trial, effect on fattener performance of a combined PCV2 and Mhyo piglet vaccine was compared against Mhyo vaccination only in a Mhyo positive herd with a subclinical PCV2 circulation.**
Materials and Methods: At 4 weeks of age, 168 piglets from the same farrowing batch were allocated into 84 pairs of similar piglets according to sex, weight, sow, or if not the same dam, sow parity and litter size. In each pair, one piglet was vaccinated with Porcilis® PCV M Hyo (2 ml IM) and one was vaccinated with one dose Mhyo vaccine according to the Marketing Authorisation (MA). Both groups (84 PCVM and 84 Mhyo) were exposed to natural PCV2 and Mhyo infection as evidenced by antibody seroconversion. Individual Feed Conversion Ratio (FCR), average daily weight gain (ADWG) and slaughterhouse data were registered. Furthermore, pneumonia lesions (IFIP reference method, lung scored 0 to 28), rhinitis lesions performed on snout computer tomography images (IFIP reference method, scored 0 to 20), clinical signs and mortality were also individually recorded.
Results: ADWG at the end of the nursery phase was not different between the PCVM and Mhyo groups. **PCVM group had a significantly higher ADWG than Mhyo group during the overall fattening period (+34 g/d) and for the growing (+35 g/d) and finishing (+30 g/d) periods**. FCR during the fattening period was also numerically better for PCVM group (-0.06 point). **PCVM group had significantly less pneumonia lesions than Mhyo group (mean score: 0.9 *vs* 2.2)**. Other criteria (clinical signs, mortality, rhinitis lesions and slaughterhouse data) were not significantly different between treatments.
Conclusion: In this high performing farm where PCV2 subclinically circulated in the fatteners, Porcilis PCV M Hyo vaccination significantly improved ADWG and lung lesions when compared against Mhyo only vaccination. **Return on investment (ROI) was calculated with GT Direct, an IFIP economic calculator, based on ADWG improvement and vaccination cost difference (0.8 € per pig) and indicated a 1.7 € gain for Porcilis® PCV M Hyo vaccinated pigs.**

Disclosure of Interest: None Declared

Keywords: combination vaccine, PCV2, Return on investment

O-IV-004

Interferon suppression and innate immune modulation by porcine epidemic diarrhea virus

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Introduction: Porcine epidemic diarrhea virus (PEDV) causes a highly contagious acute enteric disease of up to 100% mortality in suckling piglets. Type I interferons (IFN-α/β) are the major components of the innate immune response of hosts, and in turn many viruses have evolved to modulate the host response during infection. The viral modulation of innate immune response is poorly understood for PEDV, and the objective of the study to determine IFN antagonism of PEDV and to identify viral proteins antagonizing the IFN response during infection.
Materials and Methods: We identified MARC-145 cells as a suitable line of cells for PEDV replication. These cells were then used for study of innate immune modulation. All structural genes (S, S1, S2, E, M, and N) and all nonstructural genes (nsp1 through nsp16, plus ORF3) were individually cloned from PEDV and expressed in cells. IFN production was stimulated by an RNA analogue and the suppression of IFN production was determined by reporter assays and IFN bioassay. Western blot and immunofluorescent microscopy were used to examine degradation and subcellular distribution of proteins.
Results: PEDV was found to carry the ability to suppress the type I interferon production and subsequent expression of various antiviral proteins. A total of 10 viral proteins were identified as IFN antagonists. Of these, nsp1 was the most potent antagonist and was found to inhibit the assembly of IFN enhanceosome. The CREB-binding protein is a major component of the IFN enhanceosome and was degraded by PEDV nsp1 protein, resulting in the suppression of IFN production.
Conclusion: The degradation of CREB-binding protein by nsp1 is a novel mechanism for PEDV to suppress host IFN response. A better understanding of the role of IFN in the intestinal tract during neonatal infection will allow us to design a better strategy for PEDV control.

Disclosure of Interest: None Declared

Keywords: immune evasion, innate immunity, PEDV

Oral Abstracts - Friday 10 June 2016

O-IV-005

Alternative visualization of PRRS challenge study data facilitates examination of clinical and virological parameters over time

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Introduction: In PRRS challenge studies a large amount of data is available and is often presented in tables to demonstrate the effect of challenge. All of the effects of challenge are happening in the pigs at the same time. Still it is a challenge to visualize the simultaneous events in an optimal way. This paper aims to visualize a number of simultaneous effects of PRRSV challenge in vaccinated pigs using a time spatial moving graph.
Materials and Methods: Three blinded, vaccination-challenge efficacy studies were performed using separate cohorts of pigs (n=56 per study) to evaluate duration of immunity conferred by a new European-derived, PRRSV modified live virus (MLV) vaccine (Ingelvac PRRSFLEX EU). Pigs received either vaccine (Group 1), or placebo (Groups 2 and 3). Groups 1 and 2 were subsequently challenged with heterologous European PRRSV isolate 205817 at 20, 24 or 26 weeks post-vaccination. Group 3 was the negative challenge control. Percentage of viremic pigs, viral load in blood and rectal temperature were measured daily from day of challenge until the end of study 10 days later. Primary endpoints were gross and histological lung lesion scoring and viral load in lung tissue 10 days following PRRSV challenge. Secondary endpoints included clinical observations and average daily weight gain.
Results: Significantly lower mean histological lung lesion scores were observed in Group 1 versus Group 2 at 20 ($p=0.0065$), 24 and 26 weeks ($p<0.0001$). Mean viral load in lung tissue was significantly lower in Group 1 versusGroup 2 ($p=<0.0001$) at each observation. Cumulative viral loads in serum during days 1–10 post-challenge were significantly lower in Group 1 than in Group 2 ($p=<0.0001$) in all studies. A significant increase in average daily weight gain was observed in Group 1 compared with Group 2 at 20 weeks ($p=0.0027$) and 24 weeks ($p=0.0004$), but not at 26 weeks ($p=0.1041$).Simultaneous rectal temperature, blood viral loads, % of viremic pigs, and the end point histological lung lesions over 10 days post challenge, can be demonstrated using a time spatial moving graph.
Conclusion: These studies demonstrate duration of immunity was maintained for up to 26 weeks after PRRSV vaccination. This was confirmed by reduced gross and histological lung lesion in all studies, as well as reduced viral loads and a significantly lower number of viremic animals after artificial challenge. All improved parameters due to PRRSV MLV vaccination were effectively explained by using alternative visualisation tools.

Disclosure of Interest: None Declared

Keywords: DURATION OF IMMUNITY, PRRS , VACCINATION

O-PA-001

Monitoring nematode egg counts can replace routine anthelmintic treatment on farms with loose housed sows

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Introduction: Ascaris suum and Oesophagostomum spp. occur on many sow farms. Anthelmintic treatments are considered necessary in many countries and loose housing systems for sows may have increased the need for anthelmintic treatments.
The objective of this study was to investigate whether routine anthelmintic treatment on intensive farms with loose housed sows can be replaced by a repeated monitoring of faecal egg counts from breeding animals.
Materials and Methods: A total of 20 intensive sow farms (450 – 2500 sows) were selected from different regions of Denmark. During three years, faecal samples were collected and nematode egg counts were determined using a McMaster technique. The individual farm was examined at three to five time points during the study. At each time point, faecal samples were obtained from 10 gilts and 10 sows. Use of anthelmintic treatment of breeding animals was only performed when considered necessary based on the results from the faecal examinations. All descriptive statistical analyses were performed using Stata IC 13.
Results: Overall 20% and 9% of the faecal samples (n = 1574) were positive for A. suum and Oesophagostomum spp. One sample was positive for Trichuris suis. In positive samples the median egg count was 150 (range: 1-6200) and 300 (range: 50-5900) eggs per gram of faeces for A. suum and Oesophagostomum spp respectively.
Eight of the farms in the study decided to perform routine treatment of the herd despite collecting samples throughout the study. These farms were excluded from further descriptive statistical analysis.
A total of 47 examinations were performed on the remaining 12 farms during the study (three to five examinations per farm). Following 26% of the examinations, anthelmintic treatment of all breeding animals was subsequently performed based on the obtained egg counts. During the study period, five herds were never subjected to anthelmintic treatment while four, one and two herds were treated one time, two times and three times respectively.
Conclusion: On farms without routine use of anthelmintic treatment, it was not necessary to perform treatment following 74% of the examinations. However, anthelmintic treatment was necessary on approximately half of the farms minimum once during the three-year study period, indicating that monitoring of egg counts should be performed.
The results indicate that routine anthelmintic treatment on some intensive farms with loose housed sows can be replaced by a repeated monitoring of faecal egg counts from breeding animals combined with anthelmintic treatments when necessary.

Disclosure of Interest: None Declared

Keywords: None

O-PA-002

Occurrence of endoparasites in piglets analysed in the frame of the PathoPig project aiming at early detection of major pig diseases in Switzerland

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Introduction: The *PathoPig* project was launched in 2014 by the Swiss Federal Food Safety and Veterinary Office (FSVO) to improve the health status of Swiss pigs and guarantee the early recognition of epizootic diseases. Farms fulfilling particular requirements (recurrent or therapy-resistant diseases of unknown origin, atypical clinical signs, increased morbidity or mortality rates and increased use of antibiotics) may submit animals for subsidized diagnosis. Based on necropsy findings, complementary diagnostic studies (e.g. histopathology, bacteriology, virology, parasitology) are selected to assess the cause of the problem. The aims of this study were to determine the parasite species infecting piglets submitted for diagnosis to the *PathoPig* project and to estimate their involvement as cause of disease.
Materials and Methods: Faecal samples from 125 suckling (*n*=39), weaned piglets (*n*=60) and fatteners (*n*=26) from 74 Swiss farms were examined by 3 coproscopical methods (sedimentation/zinc chloride flotation, SAFC and Ziehl-Neelsen staining). Samples positive for *Cryptosporidium* were further tested by PCR/sequencing to assess the species involved in the infections.
Results: In suckling piglets, *Isospora* (syn. *Cystoisospora*) *suis* was the most commonly detected parasite (12.8%), followed by *Cryptosporidium* (10.3%). Weaned piglets and fatteners were mainly infected with *Balantidium coli* (36.7 and 50%) and amoebae (26.7 and 50%), followed by *Cryptosporidium* (15 and 19.2%) and *I. suis* (6.7 and 7.7%). *Ascaris suum, Trichuris suis* and Strongylida were rarely detected (<4%) in all age categories. While *I. suis* was significantly associated with emaciation and more frequent in pigs with diarrhoea, *B. coli*, amoebae and *Cryptosporidium* were significantly more frequent in pigs without diarrhoea. *T. suis* and *A. suum* were only detected in pigs with diarrhoea, but in a low number of cases. *I. suis* infected pigs were in 30 and 40% of the cases co-infected with *E. coli* (ETEC) and *Cryptosporidium*, respectively. Six farms (8.1%) were positive for *I. suis* and 14 (18.9%) for *Cryptosporidium* infections. Interestingly, two age-related *Cryptosporidium* species were detected: *C. suis* in younger piglets (age 2-6 weeks) and *C. scrofarum* (syn. *Cryptosporidium* pig genotype II) in older ones (age 6-27 weeks). None of the pigs infected with *C. scrofarum* (*n*=8) had diarrhoea; however diarrhoea was present in 3 of 4 piglets infected with *C. suis* (co-infection with *I. suis* in 2 cases).
Conclusion: Helminth parasites were uncommon whereas protozoa were frequently detected. *I. suis* appears to be the most important parasite species associated with disease in Swiss pig farms.

Disclosure of Interest: None Declared

Keywords: Cryptosporidium, Diarrhoea, Isospora suis

Oral Abstracts - Friday 10 June 2016

O-PA-003

ASSOCIATIONS BETWEEN ASCARIS SUUM INFECTIONS AND DIFFERENT FARM MANAGEMENT PRACTICES

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Introduction: *Ascaris suum* infections remain an important threat to the pig industry. A number of studies have been done in the last years regarding diagnosis, treatment and control. The aim of this study was to investigate the prevalence of *A. suum* in Belgium and to identify associations with farm management practices.
Materials and Methods: Between January 2013 and December 2015, 162 commercial pig herds in Belgium were randomly selected and included in the study. From each herd, 10 blood samples were taken randomly from fatteners (older than 22 weeks of age). Blood samples were analyzed using Serasca® test. The OD value obtained from each animal was used to calculate the average OD value of the herd. The cutoff of the test was defined as cutoff to differentiate between positive (OD≥0.500) and negative (OD<0.500) herds. The prevalence of positive herds was calculated. In addition to the serology investigation, an anamnesis was recorded for each herd, including parameters such as type and herd size, average daily gain (ADG), as well as management factors which were considered important in the control of *A. suum* infections [production system, weaning age, presence of slatted floors, deworming strategy of fatteners and sows (number of treatments, product, route)]. A Pearson Chi-Square was performed for prevalence of positive herds *vs* type and herd size. A Pearson correlation was performed for average OD value *vs* all the parameters.
Results: In total, 47% of pig herds were positive of which 54% were farrow-to-finish herds and 36% fattening herds (P<0.05). The prevalence also varied according to the size of the farrow-to-finish herds: 57%, 31% and 64% of the herds with 0-249, 250-349 and >350 sows, respectively, were positive (*P<0.05*). Higher OD values were negatively associated (*P<0.05*) with the number of sows (herd size) (*r*=-0.230) and ADG of fatteners (*r*=-0.189). Lower OD values were positively associated (*P<0.05*) with the presence of slatted floors (*r*=0.225), presence of deworming strategy in fatteners (*r*=0.181), number of deworming treatments in fatteners (*r*=0.154) and use of ovicidal, larvicidal and wormicidal products (*r*=0.207). No other associations were found between the rest of parameters and average OD value.
Conclusion: This study revealed a high prevalence of herds infected with *A. suum* in Belgium, especially in farrow-to-finish herds when compared to fattening herds. An association between high infection level and lower growth was also observed. The herd size (<250 and > 350 sows) combined with the absence of slatted floor and lack of effective deworming strategies were associated with a high *A. suum* infection level.

Disclosure of Interest: None Declared

Keywords: Ascaris suum, management, Serasca test

O-PA004

Lagoon effluent transmits viable Ascaris eggs in an Indiana unit utilizing a shallow pit flush system to remove animal waste

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Introduction: Ascaris suum is commonly found in Indiana sow units, although sow units are becoming less contaminated with Ascaris due to modern management practices. Replacement gilts have often been a common source of new herd infections and where the replacement gilts are placed within the facility is related to the resident animals' pattern of exposure. However, in this report, the GDU had been on a quarterly deworming program using Safe-Guard and parasite monitoring over several samplings clearly documented that the gilts were not a source of new Ascaris infections. One of three sow units in a system (2200 sow breed to wean) served by this GDU consistently presented with occasional adult Ascaris worms. The other two units had been shown to be consistently negative for Ascaris based on parasite monitoring. The senior author suspected lagoon effluent in the 2200 sow unit maybe a source of Ascarid contamination. Investigative work was done to determine if lagoon effluent may be transmitting Ascaris eggs back into this unit.
Materials and Methods: In July 2015 fecal samples from 190 individual females in the 2200 sow unit and a lagoon effluent sample were submitted to Myers Parasitology Services for analysis. In October, 2015 a second submission of 200 individual fecal samples plus lagoon effluent was submitted. All fecal samples were tested using the Wisconsin Centrifugal Sugar Flotation method. The effluent was tested using a combination of passive sedimentation, centrifugal concentration in water and then analyzed using the Wisconsin Method.
Results: In July 2015 the lagoon effluent sample was found to contain Ascarid eggs and the individual samplings revealed that 1 of the 190 samples tested positive for Ascaris eggs. In October 2015 Ascaris eggs were again found in the lagoon effluent and 3 of the 203 individual samples tested positive. In October the Ascaris eggs found in the effluent were carefully examined for viability based on egg appearance and the presence of an intact larva inside the Ascaris egg shell. The count of viable eggs in the gallon of lagoon effluent submitted for analysis contained 66 viable Ascaris eggs.
Conclusion: The finding of viable Ascaris eggs in lagoon effluent is noteworthy and supports the hypothesis that lagoon effluent may be a source of Ascaris transmission in units employing a shallow pit flush system of animal waste removal. Production sites using this or similar flush systems should consider testing lagoon effluent for the presence of viable Ascaris eggs. Ascarid contamination in lagoon waste water could be a source of previously overlooked Ascarid contamination in swine production systems.

Disclosure of Interest: None Declared

Keywords: ascaris eggs, lagoon waste, worm

O-PA-005

Novel insights in the prevalence of Ascaris suum in piglets

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Introduction: *Ascaris suum* is a widespread parasitic nematode that causes infection in fattening pigs. The clinical symptoms are mostly vague and unspecific.
Based on the high prevalence of infections with *A. suum* observed in fattening pigs, the questions arise whether exposure to *A. suum* mainly occurs in the fattening units or earlier on in farrowing and nursery units and whether serology could be used to detect exposure to *A. suum* in piglets. To achieve this, 3 different serological tests were evaluated on serum samples from artificially infected piglets.
Materials and Methods: To address this question an artificial infection of seronegative piglets took place. 3-week-old piglets were randomly divided into 4 groups of 10 animals and received a daily infection of 10, 100 and 500 eggs/day during 7 consecutive weeks. One group served as a negative control group. Blood was collected on a weekly base. Sera were individually analysed on three different ELISA's based on the recognition of several *A. suum* antigens: Haemoglobin Ag purified from the pseudocoelomic fluid from adult *A. suum* worms, As-12 Ag present on the surface of the infective L3 larvae and the complete extract of L3 larvae migrating through the lungs.
Results: A clear seroconversion was visible on the ELISA test using complete homogenate of the lung stage larvae, whereas no to low seroconversion was detectable with the Serasca test and the As-12 based ELISA. Serological results obtained in piglets during the nursery period indicated that at the time of weaning (week 1) some piglets were already highly positive for anti-*Ascaris* antibodies and that these antibody levels correlated significantly with anti-*Ascaris* antibody levels in the respective sows, suggesting maternal transfer. This was further supported by the fact that anti-*Ascaris* antibody levels in the piglets further dropped till 5 weeks post weaning after which the animals remained seronegative. Further analysis of anti-*Ascaris* antibody levels in 6 sows and 4 of their piglets (at the time of weaning) showed that the antibody levels in the piglets correlated significantly with the levels in their respective sows, suggesting the protective nature of this maternal immunity. The mechanism and duration of the maternal immunity and the potential implications it has on the deworming strategies of both sows and piglets are currently being further investigated.
Conclusion: This study shows that an ELISA test based on the recognition of migrating L3 larvae can be used as a tool to detect an early A. suum infection in piglets. In a next phase of this study it is necessary to determine the sensitivity and specificity of this test more accurately in order to obtain a cut-off value.

Disclosure of Interest: None Declared

Keywords: Ascaris suum, ELISA, weaned pigs

Oral Abstracts - Friday 10 June 2016

O-MYC-001

Mycoplasma hyopneumoniae gilt aclimatation: investigating the optimal seeder-to-naïve ratio for successful natural exposure

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Introduction: *Mycoplasma hyopneumoniae* is a swine respiratory pathogen that leads to compromised animal welfare and economic losses in finishing pigs. The lung infection can last up to 7 months and infected animals can become asymptomatic carries capable of infecting other pigs. The pathogen is mainly transmitted by direct contact, and vertical transmission is recognized as risk factor for prevalence of *M. hyopneumoniae* at weaning. It is known that colonized suckling piglets are considered initiators of the spread of *M. hyopneumoniae* after weaning, infecting susceptible pen mates. Since the situation of replacement gilts negative to *M. hyopneumoniae* being introduced into commercial farms endemically positive for the pathogen is still forceful, acclimation measures need to be taken into consideration. An alternative method for gilt acclimation is the controlled natural exposure of naïve gilts to shedding animals. Therefore, the aim of this study was to evaluate the optimum seeder-to-naïve gilt ratio in a 4-week period for successful exposure to *M. hyopneumoniae*.
Materials and Methods: Sixty gilts were divided in two groups, 21 2-week old seeder gilts were inoculated with *M. hyopneumoniae*, and 39 age-matched naïve gilts were exposed to seeders during a 4-week period. The exposure was performed by dividing the gilts into six groups of 10 with different ratios of seeder-to-naïve, from 1:9 until 6 seeders and 4 naïve gilts. Laryngeal swabs, oral fluids and blood samples were collected from all gilts prior to, during, and after inoculation and exposure. Infection in seeders was confirmed by development of clinical signs, seroconversion post-inoculation, and detection of *M. hyopneumoniae* genetic material. Naïve gilts were considered positive after 4 weeks of exposure if *M. hyopneumoniae* was detected on bronchial swab or fixed lung tissue.
Results: Thirty three percent (3/9) naïve gilts were positive in the 1:9 ratio, 75% (6/8) in 2:8, 28% (2/7) in 3:7, 33% (2/6) in 4:6, 80% (4/5) in 5:5, and 100% (4/4) in the 6:4 ratio. Considering the results on laryngeal swabs, *M. hyopneumoniae* genetic material was detected on all naïve gilts in the ratio 5 seeders and 5 naïve gilts, implying that this ratio can possibility be used to achieve exposure of all gilts in the group in longer periods of exposure. The estimated transmission rate (β) and expected probability of infection (ψ) were 1.28 per pig/week and 0.6, respectively.
Conclusion: Six seeders were required in a group of 10 gilts for successful exposure to *M. hyopneumoniae* in a 4-week exposure period. Nevertheless, a ratio of 1:1 could be possibly used in a less conservative approach and longer exposure periods.

Disclosure of Interest: None Declared

Keywords: Enzootic pneumonia, Gilts aclimatation, Natural exposure

O-MYC-002

Occurrence of Mycoplasma hyorhinis infections in fattening pigs and association with clinical symptoms and pathological lesions of Enzootic Pneumonia

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Introduction: Respiratory disorders in fattening pigs are of major concern worldwide. Particularly Enzootic Pneumonia (EP) remains a continuing problem for the pig industry leading to economically relevant losses due to retarded growth, poor feed conversion and predisposition to bacterial co-infections in affected pigs. This chronic respiratory disease is primarily caused by *Mycoplasma* (*M.*) *hyopneumoniae.* However, more recently it was hypothesised that *M. hyorhinis* can also cause similar lung lesions.
Materials and Methods: To investigate the relevance of *M. hyorhinis* as a cause of pneumonia in fattening pigs 10 farms in Switzerland (considered free of EP) and 20 farms in Germany (regarded as endemic for EP) with a history of chronic and/or recurrent respiratory diseases were included in the study. During a one-time farm visit the coughing index was determined in the batch of oldest fattening pigs in each farm before submission to slaughter. In total, 1375 lungs from these pigs were collected at the abattoir and individually scored. Furthermore, 600 lungs with, if present, indicative lesions for EP (purple to grey areas of tissue consolidation in the cranio-ventral lung lobes) were tested for mycoplasma species by culture and by real-time PCR for the presence of *M. hyorhinis* and *M. hyopneumoniae*.
Results: In total, 15.7% of the selected lungs were tested positive for *M. hyorhinis* by real-time PCR. The prevalence of *M. hyorhinis* was 10% in Switzerland and 18.5% in Germany and differed significantly between these two countries (p=0.007). *M. hyorhinis* was detected significantly more often in pneumonic lungs (p=0.004) but no significant association was found between *M. hyorhinis* and the coughing index or the *M. hyopneumoniae* status of the pig. *M. hyopneumoniae* was detected in 0% and 78.5% of the selected lungs in Switzerland and Germany, respectively.
Conclusion: Real-time PCR was almost twice as sensitive compared to the cultural approach and should therefore be used in routine diagnostics of mycoplasma species. We found no evidence that *M. hyorhinis* alone can lead to similar lung lesions as obtained by an infection with *M. hyopneumoniae* in fattening pigs. In addition, a simultaneous infection with both *M. hyorhinis* and *M. hyopneumoniae* did not aggravate the observed lung lesions. Moreover, the presence of *M. hyorhinis* showed no clinical effect in terms of coughing at least at the end of the fattening phase. However, different levels of virulence of *M. hyorhinis* isolates as well as interactions with viral pathogens like porcine reproductive and respiratory syndrome virus or porcine circovirus type 2 were reported in the literature and need to be further investigated.

Disclosure of Interest: None Declared

Keywords: coughing, Enzootic Pneumonia, Mycoplasma hyorhinis

O-MYC-003

Assessing the use of oral fluid samples for identification of Mycoplasma hyopneumoniae using a quantitative Real-Time PCR assay

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Introduction: *Mycoplasma hyopneumoniae* (*Mhyo*) is the primary pathogen of enzootic pneumonia, a chronic respiratory disease in pigs. *Mhyo* is also considered as one of the main agents contributing to the porcine respiratory disease complex. Diagnosis of *Mhyo* is key for the control of this pathogen. Evaluation of clinical signs, slaughterhouse lung checks, serological analysis or direct identification of *Mhyo* by PCR can be part of the diagnostic approach. It has been showed recently that tracheo-bronchial swabbing (TBS) is one of the best sampling techniques for direct identification of *Mhyo*. To our knowledge, studies investigating the use of oral fluids (OF) for direct identification of *Mhyo* are scarce. The aim of this study was to compare TBS and OF sampling for direct identification of *Mhyo* by quantitative Real-Time PCR assay.
Materials and Methods: Two farrow-to-finish farms positive for *Mhyo* were selected for this study, both located in Brittany, France. In *farm 1*, sucklers were sampled just before weaning (21 days old) and weaners around 70 days of age. In *farm 2*, only weaners were sampled at 33 days of age. For each farrowing or post-weaning pen, OF samples were collected using a cotton rope (Ø0.8 cm, 30 min/pen) and for each piglet, TBS were collected individually. Direct identification of *Mhyo* was then performed using a quantitative Real-Time PCR assay (Marois *et al.*, 2009).
Results: In *farm 1*, at 21 days of age, all the piglets sampled (n=114) were negative for *Mhyo* by TBS. The OF samples collected in the corresponding farrowing pens were also negative. Using TBS at 70 days of age, 19 pigs out of 100 were positive for *Mhyo*, results ranging from $5.5x10^2$ to $5.1x10^7$ CFU/ml. At the pen level, 4 pens were negative and 2 pens were positive using TBS, with 10 pigs out of 16 and 9 pigs out of 27 being positive respectively. All OF samples were negative for *Mhyo*. In *farm 2*, at 33 days of age, 21 piglets out of 33 and 2 piglets out of 18 were *Mhyo* positive using TBS in two different pens, results ranging from $5.6x10^2$ to $4.5x10^3$ CFU/ml. The corresponding OF samples of these two pens were negative.
Conclusion: Contrary to TBS, pooled OF sampling did not allow direct identification of *Mhyo* using a quantitative Real-Time PCR assay. This difference in sensitivity could be explained by the individual sampling performed for TBS compared to OF samples taken at pen level and by the sampling material/site for *Mhyo* (saliva/mouth versus mucus/lower respiratory tract). DNA damage by enzymes in the saliva, low yield of DNA extraction from saliva and/or the presence of PCR inhibitors in the saliva can play a role in the lower sensitivity of OF samples compare to TBS.

Disclosure of Interest: None Declared

Keywords: Mycoplasma hyopneumoniae, Oral fluids, PCR

O-MYC-004

Proposed management for Mycoplasma hyopneumoniae gilt acclimation, a view point

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Introduction: *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) gilt acclimation is an important management aspect in most swine farms. In this document we propose a timeline for acclimation of incoming negative females into positive herds, and comment on the epidemiological aspects in which this approach is based. *M. hyopneumoniae* is one the most important contributors to respiratory disease in pigs, causing chronic infections, and predisposing pigs to other diseases of viral and bacterial origin. Reports from pig producers and practicing veterinarians, and data from the scientific literature have demonstrated the effect of gilt influx management practices, and have helped understanding the potential implications of introducing naive gilts into an endemically infected farm. Therefore, in this document we propose a timeline for acclimation of negative gilts that are entering a *M. hyopneumoniae* positive farm.
Materials and Methods: Based on the premises that *M. hyopneumoniae* shows a long persistence and infectious period and a low transmission rate, the ideal scenario for gilt acclimation would be to receive negative gilts at a young age and way in advance, allowing for an effective exposure to the microorganism, development of disease, and recovery from the infectious period that takes place before the gilt's first farrowing. Choosing first farrowing as the reference for desired stop of bacterial shedding is based on the repeated demonstration of the dam's influence on piglet's colonization with this bacterium, and the suggested effect of pre-weaning *M. hyopneumoniae* prevalence on disease severity during the growing and finishing phases of production.
Results: This proposed timeline for gilt acclimation will be to start the acclimation/exposure (natural infection) process of the young females at no later than 50 days of age (doa), and to provide a safe and effective introduction of those gilts to infected sows of the recipient herd. Due to the slow nature of *M. hyopneumoniae* infections, vaccinated gilts will start developing infection and shedding of the microorganism in the following 4-6 weeks after exposure. Assuming that shedding lasts for less than 240 days, gilts would need to be confirmed infected by 100 doa, allowing for total recovery and lack of shedding by the time the first farrowing occurs (approximately 350 doa).
Conclusion: This approach and timeline would allow for gilts go through the infectious process, and recover in a safe and controlled manner, before they become a source of infection for their progeny. Overall, control of *M. hyopneumoniae* could be achieved by warranting uniform and controlled exposure of the reproductive herd.

Disclosure of Interest: None Declared

Keywords: Gilt acclimation, Mycoplasma hyopneumoniae, Reproductive herd management

Oral Abstracts - Friday 10 June 2016

O-MYC-005

Genetic diversity of Mycoplasma hyopneumoniae at herd level and its impact on the severity of Mycoplasma-like lesions in slaughter pigs.

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Introduction: Multiple Locus Variable Tandem Repeat Analysis (MLVA) of *M. hyopneumoniae* isolates revealed that multiple genetically different strains may be present in one herd. Studies examining the possible impact of simultaneous or subsequent infections with multiple *M. hyopneumoniae* strains on the severity of lung lesions in herds vaccinating against *M. hyopneumoniae* are scarce, although a possible impact was suggested (Villarreal et al., 2009; Vranckx et al., 2011). The aim of this study was therefore to investigate the possible impact of the presence of multiple strains/clusters of *M. hyopneumoniae* in herds vaccinating the piglets against *M. hyopneumoniae* on the severity of lung lesions in fattening pigs at slaughter.
Materials and Methods: Ten closed herds, vaccinating against *M. hyopneumoniae* were selected. Within each farm, 20 bronchoalveolar fluids (BALf) were collected randomly from 3 batches of slaughter pigs and tested for presence of *M. hyopneumoniae* DNA with nPCR. Severity of *Mycoplasma*-like lesions (MLL) of the lungs were scored (Morrison et al., 1985) at the slaughterhouse. The nPCR positive samples were submitted to MLVA with following variable number of tandem repeat's (VNTR's): p146, h1, h2, and p97. A strain was defined as an MLVA-type with at least one VNTR locus/loci mutation difference(s) to another MLVA-type. A cluster was defined as a closely related group of strains which evolved from each other by only one VNTR locus mutation. The association between MLL, Ln-transformed (outcome variable) and the number of strains or clusters in each batch of pigs per farm as independent variables was determined using two (strain and cluster) linear mixed regression models. The number of strains/clusters found in a batch per herd were assigned to following categories: 1) only one strain or cluster, 2) 2-6 strains or 2-4 clusters, 3) ≥ 7 strains or 6 to 7 clusters found per batch per herd.
Results: From the BALf, 495 of 600 (82.5%) samples tested positive for *M. hyopneumoniae* with nPCR. In the 10 herds, 135 different *M. hyopneumoniae* strains and 46 clusters were found. In total, 3820 lungs were evaluated. The average MLL of all lungs was 4.59% (min. 0%; max. 85.7%). The MLL was higher on farms with 2-6 strains (4.0%; P =0.067) and ≥ 7 strains (5.5%; P = 0.014) than on farms with 1 strain (0.8%). No significant associations were found between the number of clusters per herd and the MML.
Conclusion: There was a high diversity at strain and cluster level in these farms. A higher number of strains was associated with more severe MLL, but this was not the case for the number of clusters found on the farm.

Disclosure of Interest: None Declared

Keywords: genetic diversity, Mycoplasma hyopneumoniae, Severity Mycoplasma-like lung lesions

O-MIS-001

Why do neonatal piglets get diarrhoea?

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Introduction: Diarrhoea during the first week of life is a big concern in many Danish pig herds - not least since the suspected introduction of "New Neonatal Diarrhoea Syndrome." The study investigates pathogens and management-factors associated with these problems.
Materials and Methods: A total of 107 herds including 55 Case-herds with diarrhoea during the first week of life and 52 Control-herds without diarrhoea as a herd problem filled in questionnaires related to housing, feeding and management strategies. Within these herds, 67 herds (37 Case-herds and 30 Control-herds) submitted piglets (4 with diarrhoea and 2 without) for detection of rotavirus A, Clostridium difficile, Clostridium perfringens beta2-genes and E.coli virulence genes (LT, STa, STb, EAST-1, AIDA-1, F4, F5, F6, F18, F41). All analyses were carried out by q-PCR. Tests for E.coli virulence genes were carried out on herd level (on pools of E.coli isolates from all 6 piglets). Chi-square tests were used to test significant differences. In the analyses of management factors, generalized linear models that took plausible interactions into account were constructed.
Results: Management-factors associated with diarrhoea were;
- Sow-health* (OR 6 [95% CI 1.4 – 39.4], P=0.01)
- Neglecting drying out of farrowing crates after washing (OR 4 [95% CI 1.3 – 12.3], P=0.01)
- "Manual"** heating systems (OR 2 [95% CI 0.9 – 4.3], P=0.1) and
- Liquid sow-feeding (OR 2 [95% CI 0.9 – 4.8], P=0.1).

*: Subjectively assessed by herd vet.
**: "Manual" meaning by straw or wood-heating as opposed to oil-heating or heat-pump.
Diarrhoea was associated with the detection of rotavirus A. 22% of diarrhoeic piglets vs. 5% of non-diarrhoeic piglets were positive (P<0.0001).
Three E.coli virulence factors seemed to be associated with diarrhoea. The combination AIDA-1, EAST-1 and STb was seen in 27% of Case-herds vs. 13% of Control-herds (P=0.2).
Diarrhoea was not associated with the detection of C.difficile or the detection of C.perfringens beta2-genes. 64% of diarrhoeic piglets vs. 61% of non-diarrhoeic piglets. were C. difficile positive. Beta2-genes were detected in 91% of diarrhoeic piglets and 91% of non-diarrhoeic.
Conclusion: According to this study, important pathogens in neonatal diarrheoa in Danish herds are rotavirus A and probably E.coli carrying the virulence-genes AIDA-1, EAST-1 and STb. Neither Clostridium difficile nor clostridial Beta2-genes seem to be associated with neonatal diarrhoea.
Poor sow health, neglecting drying out farrowing crates, unstable heating and liquid feeding were management-factors associated with diarrhoea.

Disclosure of Interest: None Declared

Keywords: Neonatal diarrhoea, Risk factors, Rotavirus A

O-MIS-002

Evaluation of female pigs dosed with Improvest® and raised with Improvest males in a Canadian swine production setting

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Introduction: Market weight is an important economic factor in commercial hog operation profitability, often resulting in animals being fed longer to reach increased market weights. Suppressing estrus onset in older market gilts may reduce unwanted behaviors and increase time feeding, potentially minimizing stress and performance differences especially in mixed gender housing. This study examined the effectiveness of 2 doses of Improvest on suppression of hormones and reproductive organ maturity, as well as production and carcass effects in female pigs housed with Improvest males.
Materials and Methods: 168 healthy females, housed in 28 pens with 6 female and 6 male pigs each, were randomized to treatment: Improvest female (TF) and Improvest male, or Control female (CF) and Improvest male. Improvest animals were dosed on the same schedule: 1st dose administered 12 days after entry into grow-finish at 10 weeks of age, and 2nd dose at 16 weeks of age (7.5 weeks before slaughter). CF received 2 mL of sterile saline on the same schedule. TF and CF blood samples were collected twice before the 2nd dose and once/week after the 2nd dose, and assayed for estradiol and progesterone. CF and TF grow-finish start and finish live weights and pen level feed intake were recorded. 56 CF and 55 TF females had carcass evaluation performed and reproductive tracts assessed. Carcasses were assessed by measuring primal cut weights, loin marbling and Minolta colour scores. Treatment impact on sera hormone levels post-2nd dose and reproductive tracts at slaughter were the primary study endpoints.
Results: TF had lower mean sera estradiol than CF for three time periods post-2nd injection (1.45 vs 1.84; 1.71 vs 2.02; 2.30 vs 2.82 pg/mL, P<0.05). Mean sera progesterone was lower in TF than CF for two of three time periods post-2nd injection (0.028 vs 0.041; 0.027 vs 0.040 ng/mL, P<0.05). CF had heavier reproductive tracts (90.0 vs 28.2 g, P<0.001) and heavier ovaries (9.1 vs 3.7 g, P<0.001) than TF. All CF had visually active ovaries compared to 4% of TF (P<0.001). Pens with TF consumed 0.1 kg feed/day more than pens with CF (P<0.001), although there was no significant difference in final liveweight (CF 116.3 vs TF 118.8 kg, P=0.29). TF had heavier untrimmed bellies (5.40 vs 5.07 kg, P=0.03) and numerically heavier squared bellies (4.56 vs 4.35 kg, P=0.10) than CF.
Conclusion: TF had lower estradiol and progesterone concentrations and less reproductive tract development than CF. TF had higher feed intake and heavier belly weights than CF, with no differences in other carcass parameters. Improvest use in gilts can suppress estrus activity, potentially improving management of mixed gender production.

Disclosure of Interest: L. Van De Weyer Conflict with: Employee of Zoetis, R. Petracek: None Declared, C. Stahl Conflict with: Consultant for Zoetis, J. Daigneault Conflict with: Employee of Zoetis

Keywords: gilts, Improvest

Oral Abstracts - Friday 10 June 2016

O-MIS-003

Development of an agent-based model to evaluate surveillance strategies for detection of porcine reproductive and respiratory syndrome virus strains

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Introduction: Porcine reproductive and respiratory syndrome (**PRRS**) is an endemic infectious swine disease caused by an RNA virus and is responsible for considerable economic impacts. With the advent of regional control programs for PRRS, a frequently posed question relates to the design of effective surveillance strategies when the main objective is the detection of cases due to circulation of a novel PRRS virus strain. The framework to quantitatively assess such question for endemic diseases is not well described. Agent-based models (**ABM**) are a particularly appealing approach for cases in which testing a hypothesis can be challenging or unethical to perform under field conditions.
The objective of this study was to develop a stochastic ABM that would allow for the evaluation of different surveillance strategies for detection of emerging PRRS virus strains at the regional level.
Materials and Methods: The surveillance strategies evaluated in this study included (i) random sampling of fixed numbers of swine sites monthly; (ii) risk-based sampling of fixed numbers of specifically breeding swine sites (high-consequence sites); (iii) risk-based sampling of fixed numbers of low biosecurity sites (high-risk); and (iv) risk-based sampling of breeding sites that are also characterized as low biosecurity sites (high-risk/ high-consequence).
The model was developed in Anylogic®7.1.2 and captured important industry networks (production system, truck and feed) while considering the site's underlying immunity to PRRS and demographical/ biosecurity characteristics. These data were extracted for all sites participating in PRRS control programs in Ontario (n = 816). Outcomes of interest were sensitivity of surveillance systems (probability of the systems in detecting infected sites) and time to detection of the three first cases. Sensitivity analysis was performed to evaluate the impact of the expected incidence (design prevalence) on the outcomes of interest.
Results: Model results showed that surveillance system sensitivities were low and time to detection of the three first cases was long across all examined scenarios. None of the four strategies evaluated herein appeared optimal for early detection of a novel infection at the regional level considering model assumptions and the underlying population of interest.
Conclusion: Our findings suggest that modes of implementing high-risk and high-consequence risk-based surveillance strategies that are based on a site's static risk and demographic characteristics do not appear to substantially improve surveillance sensitivity in an industry that is highly interconnected. Novel strategies need to be developed for rapid detection of infectious diseases.
Disclosure of Interest: None Declared
Keywords: modelling, Porcine Reproductive and Respiratory Syndrome virus, Surveillance

O-MIS-004

Effects of time of second Improvest dose on growth and carcass yield of immunologically-castrated compared to physically-castrated barrows and gilts.

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Introduction: Improvest® (Zoetis, also known as Improvac) is an anti-GnRF vaccine for the immunological castration of male pigs for the control of boar taint. However, there is limited information on the impact of timing of 2nd Improvest dose relative to the time of harvest on growth and carcass parameters. The objective of this study was to compare different times of the 2nd on the performance of immunological-castrates (IC) relative to physical-castrates (PC) and gilts (G).
Materials and Methods: The study was carried out as a RCBD with 12 treatments: Treatments 1 to 4, IC given 2nd Improvest dose at wk 14, 16, 18, and 20 of age, respectively; harvested at wk 24 of age; Treatments 5 and 6, IC given 2nd dose at wk 20 of age; harvested at either wk 26 or 28 of age; Treatments 7 to 9, PC harvested at either wk 24, 26, or 28 of age, respectively; and Treatments 10 to 12, G harvested at either wk 24, 26, or 28 of age, respectively. A total of 288 pigs were housed in groups of 3 (8 groups/treatment). Diets were formulated for requirements of intact males; pigs had *ad libitum* access to feed. Pigs were harvested at a commercial facility and hot carcass weight was measured.
Results: Growth performance results are from 29.3±3.30 kg body weight to respective harvest time (134.3±10.4, 144.3±12.1, and 153.7±11.9 kg BW for wk 24, 26, and 28 of age, respectively). G had lower ($P \leq 0.05$) ADG and ADFI, and greater ($P \leq 0.05$) G:F than the other genders. ADG was greater ($P \leq 0.05$) for all IC than PC treatments (1023, 1015, 1003, 1048, 1039, 975, 971, 958, and 921 g for Treatments 1 to 9, respectively; SEM 15.4). ADFI was lower ($P \leq 0.05$) for Treatment 3 and 4 than the other IC and PC treatments (2.93, 2.97, 2.77, 2.77, 2.93, 2.93, 2.89, 2.98, and 2.96 kg for Treatments 1 to 9, respectively; SEM 0.054). G:F was greatest ($P \leq 0.05$) for Treatment 4 compared to the other IC and PC treatments (0.350, 0.343, 0.363, 0.378, 0.355, 0.333, 0.336, 0.323, 0.311 for Treatments 1 to 9, respectively; SEM 0.0051). Carcass yield was similar ($P > 0.05$) for PC and G. Increasing the time from 2nd dose to harvest in IC harvested at wk 24 of age tended ($P = 0.08$) to increase carcass yields (73.7, 73.9, 73.0, and 73,1% for Treatments 1 to 4, respectively; SEM 0.32). Carcass yield was greater ($P < 0.001$) for IC given the 2nd dose at wk 20 and harvested at wk 28 compared to wk 24 with those harvested at wk 26 being intermediate (73.1, 74.1, and 74.8% for Treatments 4, 5, and 6, respectively; SEM 0.29).
Conclusion: These results suggest that the optimum economic approach to producing IC will depend on the balance between the reduction in growth performance and the improvements in carcass yield from increasing the time between the 2nd Improvest dose and harvest.

Disclosure of Interest: C. L. Puls Conflict with: supported by Zoetis, M. Ellis Conflict with: supported by Zoetis, M. A. Mellencamp Conflict with: employee, W. Beckman Conflict with: employee, A. L. Schroeder Conflict with: employee, F. K. McKeith Conflict with: supported by Zoetis, A. Aldaz Conflict with: employee

Keywords: carcass quality, immunological castration, Improvest

O-MIS-005

The prevalence and trends of economically important porcine production diseases in Northern Ireland

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Introduction: Abattoir surveillance data is an important tool for disease monitoring and the detection of animal welfare conditions. The Northern Ireland voluntary pig health scheme, co-ordinated by Pig Regen, has recorded the presence of eight different macroscopic lesions detected in the pluck and on the skin of slaughter pigs. These economically significant sub-clinical infections therefore can be considered to be performance indicators.
Materials and Methods: A total of 90,938 pigs from 1145 herds were subject to lesion monitoring over a 2-year period from 2013-2014. Average prevalence data was obtained over a total of 10 seasons from 2008 to 2012 to allow for analysis of trends over time. A descriptive analysis of the data set was compiled and used to calculate the observed prevalences and trends associated with each lesion.
Results: Milk spot lesions were the most prevalent condition at 16% amongst slaughter pigs. The most prevalent herd-level lesion was pleurisy at 73%. Enzootic pneumonia-like lesions were found to be associated with pleurisy ($p<0.001$), pericarditis ($p<0.05$), lung abscesses ($p<0.001$) and papular dermatitis ($p=0.083$). Pleurisy lesions were similarly correlated with enzootic pneumonia, pericarditis ($p<0.001$) and pleuropneumonia ($p=0.0034$), and correlations were also found between pleurisy and tail bite ($p=0.037$). In addition to pleurisy, pleuropneumonia lesions were also associated with lung abscesses ($p<0.001$). Milk spot lesions were associated with papular dermatitis ($p=0.0052$). A negative trend was observed in the prevalence of enzootic pneumonia-like lesions ($p<0.001$) over time and in the prevalence of lung abscesses ($p=0.007$). A positive trend was found for pericarditis lesions with an increase in prevalence observed over time ($p=0.003$).
Conclusion: The prevalences of respiratory lesions amongst pigs in NI are similar to those observed for the rest of the UK, with the exception of enzootic pneumonia, which is significantly lower in prevalence. The high prevalence of milk spot lesions indicates that parasitic infection remains a problem within the pig sector in NI. A close association between respiratory lesions was expected due to their likely shared causal factors, as was the relationship observed between lesions caused by parasitic infection. A decrease in enzootic-pneumonia-like lesions and lung abscesses has been observed over time, which may be attributed to a greater uptake in vaccination against *Mycoplasma hyopneumoniae*. This data can be used to provide pig processers and producers with detailed herd health information that can potentially contribute to reduced economic losses and lead to higher animal welfare standards.

Disclosure of Interest: None Declared

Keywords: Northern Ireland, Porcine production diseases, Prevalence

Poster Abstracts

Poster Abstracts

Bacteriology and Bacterial Diseases
ACTINOBACILLUS

PO-PF3-097

Oral fluid ELISAs for differentiated detection of antibodies against Actinobacillus pleuropneumoniae serotypes 2, 6 and 12.

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Introduction: In the Danish SPF-system surveillance on *Actinobacillus pleuropneumoniae* (AP) is based on the ability to differentiate between different serotypes of AP. Decisions on trading and moving swine are often relying on the infection-status obtained from serological examinations concerning different AP serotypes. The ELISAs used for this have until recently only been validated for serum. In this project, three in-house indirect ELISAs for AP serotypes 2, 6 and 12 were optimized for analysis of oral fluid (OF) samples. Furthermore an in-house mix-ELISA for detection of antibodies against all three serotypes simultaneously was optimized for analysis of oral fluid.
Materials and Methods: Samples for validation were obtained from Danish pig herds in cooperation with DIANOVA and veterinary practitioners from Odder Svinepraksis. Pigs that were seropositive to Ap 2, 6, or 12 as well as negative pigs, were sampled. Oral fluid pen pools were collected by hanging a rope in selected pens. For comparison, blood was drawn from all pigs in each OF-sampled pen. A total of 1317 sera and 148 matching OF pools were sampled, representing pigs from 8-130 kg. All sera were tested in our in-house AP ELISAs used in the SPF surveillance, and these results served as gold standard in the validation of the optimized OF-ELISAs. In the statistical analyses, an AP-positive pen was defined as a pen with at least 50% pigs positive in the serum-ELISAs. The overall performance of the OF-ELISAs was evaluated by ROC curves for different dilutions of the oral fluid.
Results: When setting pen specificity to 0,97 for all tests, the performance of the AP OF-ELISAs was calculated under the assumptions that sample size would be 10 OF-samples (i.e. 10 pens) and the within herd prevalence of the AP-infection would be 0,2. Calculated herd sensitivities were 0,88 in the AP2 OF-ELISA, 0,91 in the AP6 OF-ELISA, 0,54 in the AP12 OF-ELISA and finally 0,76 in the AP-MIX OF-ELISA. Sensitivity of oral fluid antibody-tests is generally lower than that of serum-tests due to physical characteristics of OF-samples such as low antibody concentration and contamination. Given the fixed pen specificity of 0,97, if you take 10 rope samples in one herd, the herd specificity will be 0,74 for all four OF-ELISAs. The herd specificities were calculated based on the rather pessimistic assumption, that false positives are not aggregated on herds. Biologically this is not the case, so real life specificities are expected to be higher.
Conclusion: We have developed ELISAs for detection of antibodies against *Actinobacillus pleuropneumoniae* in oral fluid that can be used as monitoring test alternatives to the serum based diagnostics of AP serotype 2, 6, and 12 in pig herds.

Disclosure of Interest: None Declared

Keywords: Actinobacillus pleuropneumoniae, ELISA, oral fluid

Bacteriology and Bacterial Diseases
ACTINOBACILLUS

PO-PF3-099

Pharmacokinetics of tildipirosin in pig tonsils

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Introduction: Tildipirosin (TD) is a semi-synthetic tylosin analog that has been approved for the treatment of respiratory diseases in pigs and cattle. This macrolide is rapidly absorbed and extensively distributed to the site of respiratory infection. Thus, lung, mean TD concentrations were characterized by a peak on day 1 and a slow decline until 17 days after administration. On the other hand, pigs can become asymptomatic carriers of *Actinobacillus pleuropneumonia*e (APP) in their tonsils for long periods. In the literature, antimicrobial treatments have been used to eradicate APP from tonsils. Thus, the goal of this study was to quantify the TD penetration in tonsils and to characterize its pharmacokinetic profile at the registered dose (4 mg of TD/Kg bw) as a first step to check the potential use of this molecule for eradication of APP from tonsils in carrier animals
Materials and Methods: Forty-eight 2-month-old clinically healthy hybrid pigs were randomly divided into six groups (control, T1, T2 (1), T2(5), T2(10) and T2(15)) of eight animals. T1 and T2 groups received a dose of 2 and 4 mg of TD/Kg bw in one shot respectively and the control group received 2 mL of saline solution. The animals were sacrificed by intravenous administration of pentobarbital sodium twenty four hours after finishing the treatment for the control, T1 and T2(1) groups whereas animals of T2(5), T2(10) and T2(15) were sacrificed at 5, 10 and 15 days, post-treatment, respectively. Tonsils and blood samples were taken at necropsy to quantify the concentration of TD using a high-performance liquid chromatography (HPLC) with tandem mass spectroscopy detection (LC/MS/MS). Plasma and tonsil PK parameters were determined with a non-compartmental analysis
Results: Average tildipirosin plasma (ATPC) and tonsil (ATTC) concentration for the control, T1 and T2(1) group increased significantly ($p<0.05$) in a dose-dependent manner. On the other hand, the concentration in plasma was always significantly lower ($p<0.05$) than in tonsil for the groups treated with 4 mg of TD/kg bw at 1, 5, 10 and 15 days post-treatment, while the maximum concentration of tildipirosin in tonsil was observed at 1 day post-administration with a gradual decrease until 15 days post-administration. In particular, mean tonsil tildipirosin concentration is above the MIC_{90} (2 µg/mL) for APP for about 5 days
Conclusion: The data demonstrate that tildipirosin is present in tonsils above MIC_{90} for APP for an extended period of time. Further *in vivo* studies with carrier pigs will need to be pursued to show that the concentrations are effectively able to eliminate APP from tonsils. The results provide a good basis to continue work in this area

Disclosure of Interest: None Declared

Keywords: Tildipirosin, APP, tonsil

Bacteriology and Bacterial Diseases
ACTINOBACILLUS

PO-PF3-055

Prevalence of Actinobacillus pleuropneumoniae serovars in Poland

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Introduction: Porcine pleuropneumonia, caused by Actinobacillus pleuropneumoniae (App), is worldwide in its distribution and has caused severe economic losses in most pig-rearing countries. Epidemiologically, serotyping is the gold standard method, with App being classified into 15 different serovars based on the presence of surface carbohydrates, principally of the capsule. Variances in the virulence between serotypes have been reported. Virulence is strongly correlated with the production and secretion of different exotoxins ApxI, ApxII and ApxIII. Knowledge of the serotypes which exist within a particular region or country is therefore important. The aim of this study was to investigate the prevalence of App serotypes in Poland.
Materials and Methods: A total of 153 *App* strains isolated from 2011 to 2015 from diseased pigs, suffering from severe respiratory signs, were tested. No more than one isolate of *App* from the same farm was included in this study. Antisera against the reference strains (kindly supplied by Dr Gottschalk, University of Montreal) were prepared using a modification of the technique described by Mittal *et al.* Isolates were serotyped using a slide agglutination method.
Results: All strains were NAD-dependent (biovar1). Among 153 strains of *App*, the reactions with rabbit polyclonal antisera were found as follow: antiserum 2 – 26.1 %, 4 – 8.5%, 5 – 7.8 %, 9 – 6.5 %, 6 – 5.9 %, 7 – 3.9 %, 11 – 2.6 %, 8 and 3 (1.3 % each), 1 and 15 (0.65 % each). Cross-reactions with antisera 4 and 7; 1, and 9 and 11, have been reported in 11.8% and 2.6%, respectively. Approximately 20 % of the isolates were untypable using the slide agglutination technique due to cross-reactions with several antisera (2-8).
Conclusion: Serotype 2 was the most common detected. Serotypes 4, 5, 9, 6 of *App* were isolated relatively frequently. Serotypes 7, 11, 8, 3, 1 and 15 occurred rarely. Classical immunological-based methods have limitations, in particular, well known, cross-reactivity between serovars 4 and 7, between serovars 1, 9 and 11, as noted in current studies. A large number of non-typable isolates (20%) requires the use of other techniques in the future like capsulation gene – based PCR.
Previous report (Tarasiuk, 1997) have recorded the presence of serotypes 9, 6, 4 and 2 with the domination of 9 serotype in Poland.

Disclosure of Interest: None Declared

Keywords: Actinobacillus pleuropneumoniae, porcine pleuropneumonia, serotyping

Bacteriology and Bacterial Diseases
ACTINOBACILLUS

PO-PF3-035

Septicemia associated with Actinobacillus suis infection in growing pigs in Korea

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Introduction: *Actinobacillus* (*A*) *suis* is a small, gram-negative rod and an opportunistic pathogen that colonizes the upper respiratory tract in pigs. Outbreaks of disease occur in high health status herds or new herds. Infected pigs may have lesions related to septicemia and/or respiratory disease, such as hemorrhagic and necrotic pneumonia. Although acute septicemia with death occurs mainly in suckling and recently weaned pigs, it can be observed in growing, finishing, and adult pigs. Here, we report a case of *A. suis* septicemia in growing pigs in a Korean domestic herd.
Materials and Methods: The case involved pigs that were 80 to 100 days old located on a two-site farm in July of 2015. Approximately 5% of growers died, and dead animals did not show any previous clinical signs. The pigs in this farm were negative for PRRS. Two dead pigs were necropsied and various organs were fixed in 10% buffered formalin. The fixed samples were embedded in paraffin and stained with H&E and Gram stains. Diagnostic confirmation was made based on the detection of pathogens by polymerase chain reaction (PCR) and bacterial culture from the lung. PCR was used to detect PRRSV, PCV2, *Pasteurella multocida*, *Streptococcus suis*, *Haemophilus* (*H*) *parasuis*, *A. pleuropneumoniae*, and *A. suis*.
Results: Grossly, the thoracic cavities in all pigs contained a large amount of dark brown fluid. The cranioventral lung lobes showed a dark red discoloration and a firm to rubbery consistency with fibrinous adhesions to the thoracic wall. Microscopically, the lungs showed severe fibrinonecrotic pleuropneumonia with bacterial colonies within the alveoli. Multiple foci of coagulation necrosis with bacterial colonies were observed both in the liver and spleen. Intralesional bacterial colonies showed gram-negative coccobacilli with a Gram stain. All pigs demonstrated positive results for *A. suis* and *H. parasuis* with PCR. In addition, NAD-independent *A. suis* was cultured from all lung samples. All of the isolates were sensitive to amoxicillin, ampicillin, cefazolin, colistin, enrofloxacin, florfenicol, ceftiofur, tiamulin, and sulfamethoxazole/trimethoprim.
Conclusion: Based on the gross, microscopic and microbiological features, these cases were diagnosed as *A. suis* infection in growing pigs. The gross features of the lung lesions may be confused with pleuropneumonia caused by *A. pleuropneumoniae* in growing and finishing pigs. Therefore, a diagnosis of *A. suis* should be confirmed by PCR or bacterial culture from affected pigs. To the best of our knowledge, this is the first report of *A. suis* infection in growing pigs in Korea.

Disclosure of Interest: None Declared

Keywords: Actinobacillus suis, growing pigs, septicemia

Poster Abstracts

Bacteriology and Bacterial Diseases
ACTINOBACILLUS

PO-PF3-115

Characterization of resistance profile of Actinobacillus pleuropneumoniae isolates in Brazil.

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Introduction: The objective of this study is to determine the antimicrobial resistance profile of ninety-nine *A. pleuropneumoniae* strains, isolated from swine lungs presenting clinical signs of the disease. Results allow veterinarians and professionals from the swine production sector to stay up-to-date with this agent's resistance profile in the country, thus contributing with the conscious use of antimicrobials in the activity.
Materials and Methods: Ninety-nine *A. pleuropneumoniae* were isolated from chest cavity and lung fragments of animals with pneumonia. For the inoculum, cultures were grown at 37°C for one day and then diluted. Fifty microliters of the inoculum were added in each Sensititre® minimal inhibitory concentrations (MIC) Plate well. MIC of the following eighteen antimicrobial agents were determined using BOPO6F MIC Plate – Sensititre®: ampicillin, clindamycin, chlortetracycline, danofloxacin, enrofloxacin, florfenicol, gentamicin, neomycin, oxytetracycline, penicillin, sulfadimethoxine, spectinomycin, cotrimoxazole, tiamulin, tilmicosin, tulathromycin, tylosin, and ceftiofur.
Results: Among the ninety-nine analyzed strains of *A. pleuropneumoniae*, all were sensitive to ceftiofur, tulathromycin, gentamicin, and tilmicosin. Low resistance levels were observed against co-trimoxazole, florfenicol, and spectinomycin (2%), ampicillin (7%), tiamulin and enrofloxacin (8%), neomycin (10.1%), and danofloxacin (12.1%).
Conclusion: The obtained results are of significant relevance for the control of swine pleuropneumonia, from the use in farms to the assessment by regulators of the need for subsidies for antimicrobials in animal production. Despite at low levels, the resistance identified in this study raises awareness with respect to the need to reduce excessive and indiscriminate use of antimicrobial agents.

Disclosure of Interest: None Declared

Keywords: A. pleuropneumoniae, antimicrobial resistance, swine pleuropneumonia

Bacteriology and Bacterial Diseases
ACTINOBACILLUS

PO-PF3-072

Occurrence of pleurisy in fattening pigs in three different epidemiological regions in Germany

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Introduction: Monitoring lung lesions at slaughter can be an efficient tool in estimating the prevalence and severity of respiratory diseases in fattening pigs. *Actinobacillus pleuropneumoniae* (App) can be associated with chronic pleurisy. German pig production can be characterized by the existence of three different epidemiological regions: Northern Germany with high pig density; Eastern Germany with fewer but larger farms and in contrast to that Southern Germany with smaller farms. The present study was conducted to estimate the appearance of pleurisy in Germany under respect of the APP serotypes on farm level.
Materials and Methods: In total 49 fattening farms (North: 20; East: 9; South: 20) with recurring respiratory disease were included in the present study. In total 4723 lungs (appr. 100 lungs per farm) were examined for the occurrence of pleurisy at slaughter. Location and extension of pleurisy was evaluated by the Slaughterhouse Pleurisy Evaluation System (SPES). SPES values from 2 to 4 qualify increased severity of dorsocaudal pleurisy. Blood samples were collected within 4 weeks after slaughterhouse checks (20/farm; in total 980) and tested for antibodies against App; 5 seropositive samples per farm (in total 204) were further serotyped.
Results: Pleurisy was evident in 2153 (45.6 %) of all examined lungs. In Northern Germany (a) pleurisy was found significantly more frequently (60.7 %) than in Eastern Germany (b; 35.7 %) and in Southern Germany (c; 34.7%) (ab; ac:$p<0.001$). Furthermore, in Northern Germany occurrence of pleurisy grades SPES 2 and SPES 3 was significantly higher than in Eastern and Southern Germany (ab; ac:$p<0.001$). The occurrence of SPES 4 was equally distributed among the regions. Farms positive for App serotype (ST) 2 had a 4 times higher (OR=3.98; $p<0.001$) risk to suffer from pleurisy. Moreover, the occurrence of further App-serotypes increased the risk for pleurisy: ST 3/6/8 (OR= 2.36); ST 10 (OR=1.82); ST 5 (OR=1.62); ST 1/9/11 (OR=1.26) ($p<0.001$). Occurrence of antibodies against ST 12 and 4/7 on farm level did not increase the risk for pleurisy.
Conclusion: Pleurisy is highly prevalent in German fattening pigs with recurring respiratory disease. The frequency of pleurisy seems to depend on the regions where pigs are housed. Farms serologically positive for App ST 2, ST 3/6/8 and ST 10 showed a significantly increased risk to suffer from pleurisy.

Disclosure of Interest: None Declared

Keywords: Actinobacillus pleuropneumoniae, pleurisy, serotype

Bacteriology and Bacterial Diseases
ACTINOBACILLUS

PO-PF3-094

Seroprevalence of Actinobacillus pleuropneumoniae and corresponding serotypes in fattening pigs in three different epidemiological regions in Germany

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Introduction: Porcine pleuropneumonia caused by *Actinobacillus pleuropneumoniae* (App) is a contagious disease still reported to cause economic losses worldwide. In recent years there was an increase of clinical App-infections in Germany. German pig production can be characterized in three different epidemiological regions: Northern Germany with a high pig density, Eastern Germany with fewer but larger farms and in contrast to that Southern Germany with smaller farms. The present study was conducted to get actual information on the seroprevalence of App and corresponding serotypes in these regions.
Materials and Methods: From March to December 2015 in total 49 fattening farms suffering from recurring respiratory disease were included in this investigation. At the end of fattening blood samples were collected (20/farm; total 980) and examined for antibodies against App using the IDEXX APX IV ELISA at the IZSLER, Italy. Furthermore, 5 seropositive samples per farm (total 204) were serotyped using the ID Vet Screen ELISA.
Results: In total 87.8% (43/49) of the farms were positive for App in the APX IV ELISA (North(a): 90%; East(b): 88.9%; South(c): 85%). The overall seroprevalence for individual animals was 62.3% (611/980). In Northern Germany significant more pigs were seropositive for App (70.5%) than in Eastern Germany (61.7%) and Southern Germany (54.5%)(ab:$p<0.05$; ac:$p<0.001$) with a mean in herd prevalence in the North of 14.1 positive animals per farm; East 12.3 and South 10.9 respectively. At farm level, antibodies against App ST 3/6/8 were detected in 71.4%, ST 5 in 57.1%, ST 2 in 55.1%, ST 12 in 51%, ST 4/7 in 32.7%, ST 10 in 30.6% and ST1/9/11 in 20.4% out of all. In 69.3% out of all farms antibodies against 3 or more different serotypes were detectable. The most frequent serotypes in individual animals were ST 2 (57.8%) and ST 3/6/8 (55.4%). Regarding ST 1/9/11 Eastern Germany has significant more seroreagents than Northern Germany (ab:$p<0.05$); regarding ST4/7 significantly more positive individuals were found in Southern Germany than in the other regions (ac:$p<0.001$; bc:$p<0.01$).
Conclusion: Farms with recurring respiratory disease showed a high App seroprevalence on herd level as well as on individual level in this investigation. Seropositive farms frequently are affected by 3 or more App Serotypes. More than 50% of all farms were positive to App ST 2 and 5.

Disclosure of Interest: None Declared

Keywords: Actinobacillus pleuropneumoniae, seroprevalence, serotype

Bacteriology and Bacterial Diseases
ACTINOBACILLUS

PO-PF3-057

Comparison of Duration of Efficacy of EXCEDE® for Swine Versus Baytril® 100 in an Actinobacillus pleuro pneumonia Challenge Model

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Introduction: *Actinobacillus pleuropneumoniae* (APP) is frequently used in bacterial respiratory disease models to investigate the efficacy of treatment regimens. This study was conducted to compare the duration of efficacy of EXCEDE for Swine and Baytril. The study was undertaken to determine whether the sustained-release formulation of EXCEDE provides a longer duration of clinical activity against the death loss and severe morbidity caused by APP.
Materials and Methods: Animals were assigned to treatments (7 treatment groups with 20 animals each) and pens according to randomized block design. One complete block of animals was housed in a single pen. On each of Days -7, -5, and -3, pigs in groups T2, T3, and T4 were administered Baytril, and pigs in T5, T6, and T7 were administered EXCEDE, whereas pigs in control group T1were administered saline on Day -3. All administrated treatments were done according to labels. On Day 0, all pigs in all study groups were challenged intratracheally with inoculum containing a strain of APP serotype 5 demonstrated to be susceptible to both Baytril and EXCEDE. Mortalities were documented throughout the study, and animals were necropsied upon death, euthanasia, and at the end of the study. On necropsy examination, individual lung lobe scores were assigned to the lung and a total percent lung score was determined. Data recording was performed by personnel masked to treatment. Statistical analyses were performed only for the primary variables, at the 5% level of significance. The entire study was conducted under veterinary supervision and was pre-approved by an Institutional Animal Care and Use Committee.
Results: Pigs in the EXCEDE treatment groups had significantly ($P \leq 0.05$) less mortality when compared with pigs in the groups treated with saline and with pigs in all groups treated with Baytril. Pigs in groups treated with Baytril on Day -3 had significantly ($P \leq 0.05$) less mortality when compared with pigs in the groups treated with saline and with pigs treated with Baytril on Day -5 and Day -7. (Day -3 – Saline 14/20, Baytril 7/20, EXCEDE 0/20) (Day -5 – Baytril 13/20, EXCEDE 0/20) (Day -7 – Baytril 14/20, EXCEDE 1/20). Animals treated with EXCEDE on all assessment days had significantly ($P \leq 0.05$) lower total lung lesion scores when compared with animals in all of the other treatment groups.
Conclusion: The APP challenge model employed in this study demonstrated that the sustained-release formulation of EXCEDE for Swine provided a longer duration of clinical activity against the death loss and severe morbidity caused by APP than did Baytril 100.

Disclosure of Interest: M. Senn Conflict with: Zoetis, B. Cowles Conflict with: Zoetis, D. Amodie Conflict with: Zoetis, A. Mueller Conflict with: Zoetis

Keywords: Actinobacillus pluropenumoniae, Draxxin, Enrofloxacin

Poster Abstracts

Bacteriology and Bacterial Diseases
ACTINOBACILLUS

PO-PF3-197

Prevalence of Actinobacillus pleuropneumoniae serotypes in swine in Germany

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Introduction: *Actinobacillus pleuropneumoniae (App)* is the etiological agent of pleuropneumonia in pigs. Although an old bacterial pulmonary pathogen, it is still relevant in the 21st century and occurs worldwide. At present, 15 serotypes have been differentiated by antisera. The distribution of serotypes involved in clinical cases in different regions varies drastically. Moreover, strains of a certain serotype may typically be highly virulent in one region while strains of the same serotype may typically be of low virulence in another. Because the available data were not current, we analyzed serotyping results of *App* strains isolated from swine in Germany within the last four years.
Materials and Methods: A total of 565 *App* isolated from swine during diagnostic examinations between 2012 and 2015 were analyzed for their serotype by multiplex PCR testing based on *apx* and *cps* genes. The distribution of serotypes of *App* were analyzed for the total population in comparison to two subpopulations of strains, i) those isolated from swine with respiratory symptoms (n = 197) and ii) those definitely isolated from the porcine lung (n = 140); and for annual variations.
Results: Serotype 2 is the predominant *App* isolated in the total population (66.4%) and in both subpopulations i) (71.6%) and ii) (61.6%). The next most prevalent *App* serogroup comprised serotypes 1, 9, and 11: 13.1% in the total population, 10.2% in subpopulation i), and 15.0% in subpopulation ii). The prevalences of serotypes 5 and 6 were lower, between 5.0% and 6.5% in all populations. The prevalence of serotype 7 was slightly higher, at 7.9% in subpopulation ii), where *App* was isolated only from porcine lungs, but it was less prevalent in the total population (2.7%) and in subpopulation i) swine with respiratory symptoms (2.5%). While the prevalence of *App* serotype 2 varied from year to year between 82 and 110 (6.3%>7.0%), there was a low-level increase in *App* serogroup 1/9/11 over the years (2012: 10 (7.6%); 2013: 14 (10.9%); 2014: 22(14.0%); 2015: 28 (18.7%)).
Conclusion: Although past and present methods for serotyping are totally different, *App* 2 remains the most predominant serotype in Germany associated with clinical respiratory symptoms and definitely isolated from the porcine lung. The considerable level of *App* recorded in German pig stocks confirms the need for constant monitoring of prevalence and serotypes, especially with respect to its central role in lung health and animal welfare. In conjunction with other bacterial and viral pathogens like Porcine Reproductive and Respiratory Syndrome virus *App* seems to be a key factor in the pulmonary health of affected pigs.

Disclosure of Interest: None Declared

Keywords: None

Bacteriology and Bacterial Diseases
ACTINOBACILLUS

PO-PF3-106

Susceptibility of Actinobacillus pleuropneumoniae isolated from swine in Italy to trimethoprim plus sulphadimethoxin

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Introduction: Antimicrobials continue to be an effective measure for the control of pleuropneumonia outbreaks in swine. The Italian ministry of health recommends not to systematically use new molecules as first line treatment (Manuale Biosicurezza e uso corretto e razionale degli antibiotici in zootecnia, Sezione suini, 2012). Thus, susceptibility to alternative treatment must be documented. Trimethoprim-sulphonamide (TMP+S) combination is recommended as first choice antimicrobial for pleuropneumonia therapy but a recent bacterial susceptibility study to this antimicrobial family reported an increasing antibioresistance level to TMP+S combination, particularly in historical collections (1994-2009) of *Actinobacillus pleuropneumoniae (*App) strains collected in Italy. This study aimed at updating the knowledge on App resistance to Trimethoprim-sulphadimethoxine (TMP+SDM) (Prazil® N Orale, Merial Italia Spa, Milano, Italy).
Materials and Methods: The study was performed using a collection of 42 *App* strains belonging to IZSLER, recently isolated in clinical outbreaks of pleuropneumonia in Italy during 2012-2014. The Minimal Inhibitory Concentrations (MIC) for TMP+SDM was determined using a broth dilution technique. In addition, the antimicrobial susceptibility testing was carried out by the agar disk diffusion test trimethoprim-sulphamethoxazole (SXT) (1.25/23.75 µg). The methods described above were performed following the CLSI guidelines.
Results: Susceptibility rate to TMP+SDM was 76% (32/42) according to the MIC results. Using the disc diffusion test the strains were classified as sensitive (40.5%), intermediate (30.9%) and resistant (28.6%) to TMP+SXT. These results show high sensitivity of recent App isolates to TMP+S combination and suggested a high efficacy of TMP+S combination against App in the field.
MIC technique can be considered a gold standard for antibiotic-resistance monitoring, in particular for some antimicrobials (such as TMP+SDM) for which disc diffusion testing could underestimate the bacterial susceptibility *in vitro*. Our results prompt to consider TMP+SDM as valuable in the treatment of pig pleuropneumoniae.
Conclusion: Susceptibility to Trimethoprim-sulphadimethoxine of a collection of *Actinobacillus pleuropneumoniae* strains isolated in clinical outbreaks between 2012 and 2014 in Italy was 76%.

Disclosure of Interest: G. Leotti Conflict with: MERIAL Italia SpA, A. Luppi: None Declared, Y. Gherpelli: None Declared, M. Dottori: None Declared

Keywords: Actinobacillus pleuropneumoniae, antibiotic susceptibility, sulfonamide

Bacteriology and Bacterial Diseases
ACTINOBACILLUS

PO-PC02-002

Efficacy of gamithromycin injectable solution (ZACTRAN®) against Actinobacillus pleuropneumoniae using an experimental challenge model in piglets

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Introduction: Actinobacillus pleuropneumoniae (App) is the primary agent of pleuropneumonia and is involved in PRDC. It critically affects economic productivity of pig farms. ZACTRAN is a novel azalide antibiotic (gamithromycin) recently licensed for the treatment of swine respiratory disease. This abstract refers to the efficacy of ZACTRAN against App in an experimental challenge model.
Materials and Methods: On Day 0 (D0), healthy 5 week-old pigs that had never been treated with antibiotics or anti-inflammatory products and were seronegative to *App* were intranasally challenged with 8.8 log10 CFU of a virulent *App* strain qualified for consistent respiratory disease induction. Pigs met clinical criteria for eligibility 4h-8h post-challenge and were then injected either with a single dose of ZACTRAN (1mL/25kg IM, n=20), or with saline (1mL/25kg IM, n=20). Clinical observations were conducted 4 hours post-treatment then once daily up to 4 days post-treatment (D4pt) and scored for: rectal temperature (0-3), demeanor (0-3), type of respiration (0-3), coughing (0-3) and body condition (0-2). Bodyweights were recorded on D-7, D0 (prior to challenge) and D4pt or prior to any unscheduled necropsy. Blood sample was collected on D-7 from each animal for *App* serotypes 1 to 12 antibody levels using a commercial kit (ID Vet Innovative diagnostics). Necropsies were performed on D4pt. The percentage pulmonary consolidation (gross involvement of lesions) was estimated. Lung tissue samples were collected from specific sites and the numbers of colony forming units per gram of lung tissue were determined. Clinicians were blinded to treatment.
Results: All pigs were serologically negative to *App* prior to challenge. Four pigs in the Saline group died or were euthanized on ethical grounds prior to scheduled necropsy with advanced challenge-related respiratory disease. In contrast, no mortality occurred in gamithromycin-treated pigs. Lung lesions were typical of acute pleuropneumonia with widespread consolidated lesions, pleural adhesions, pleural fluid and fibrin present. *App* was recovered from every lung samples from all pigs in the Saline group, compared with only 24% in the treated group. In this context, the treated pigs had significantly lower clinical scores, at all post-treatment occasions ($p<0.0001$) and over the post-challenge period ($p<0.0001$), lower lung consolidation percentages ($p<0.0001$), lower numbers of *App* recovered from lung tissue ($p<0.0001$) and better daily bodyweight gain post-treatment than those administered saline.
Conclusion: This study showed that a single treatment with gamithromycin injectable solution was efficacious for the treatment of clinical Swine Respiratory Disease associated with *App*.

Disclosure of Interest: A. Richard-Mazet Conflict with: MERIAL S.A.S., A. Pfefferkorn Conflict with: MERIAL S.A.S., D. Reddick: None Declared, C. Ramage: None Declared, P. Dumont Conflict with: MERIAL S.A.S., P. Jeannin Conflict with: MERIAL S.A.S.

Keywords: gamythromycin, injectable, macrolide

Bacteriology and Bacterial Diseases
ACTINOBACILLUS

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Efficacy of gamithromycin (ZACTRAN) in 8-week-old pigs for the treatment of Swine Respiratory clinical Disease induced by a seeder-pig challenge model

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Introduction: This study was conducted to evaluate the efficacy of a gamithromycin injectable solution (ZACTRAN®, Merial Inc.) administered intramuscularly and compared to a non-treated control group in the treatment of Swine Respiratory Disease (SRD). SRD was induced by a seeder-pig-challenge model using *Actinobacillus pleuropneumoniae* (*App*).
Materials and Methods: Eight-week-old healthy pigs, not vaccinated against any respiratory pathogens were recruited for the study. They had no history of therapeutic antibiotic usage and no history of SRD and had not been in contact with pigs of any other source. Seeder pigs were intranasally infected with *App* on Day minus 2 (D-2). Contact pigs were commingled in direct contact with seeder pigs. Contact pigs showed on D0 signs of SRD. Conditions of enrollment in the study was defined as the combination of moderate depression, moderate respiratory distress and hyperthermia. The contact pigs were randomized according to bodyweight to the following treatment groups: Controls (n=20): Saline at 0.4 mL/10 kg, Treated group (n=20): gamithromycin injectable solution at 6.0 mg/kg (0.4 mL/10 kg). Seeder pigs were euthanized after enrollment was completed.
From D0 to D7, depression and respiratory signs were scored and rectal temperatures were recorded. Starting on D3, pigs not responding to therapy were declared treatment failures then were humanely euthanized and necropsied. Surviving pigs were euthanized and necropsied on D7. Lung lobe consolidation/pneumonia lesions were evaluated grossly and scored. Individual swabs from the pneumonia lesions (or swabs of lung tissue if no lesions observed) and trachea were taken for bacteria culture. The clinician was blinded to treatment.
Clinical scores were compared using Wilcoxon's Test. Rectal temperature was analyzed through a repeated-measure mixed model. Treatment failures were analyzed using the log-rank test to compare the distributions of the number of days until the removal of animals from the study.
Results: The gamithromycin-treated pigs displayed significantly lower depression scores from D3 to D7 ($p<0.05$), lower respiratory sign scores from D4 to D7 ($p<0.05$) and lower rectal temperature ($p=0.017$). Proportion of failure to treatment in the treated group was significantly lower as well ($p<0.0001$). Overall percentages of lung lobe consolidation were significantly lower in the treated group ($p<0.05$). Culture of tracheal and/or lung samples demonstrated the presence of *App* pathogens in 100% of the animals in the control group and 35% in the treated group.
Conclusion: Under the conditions of the study, a single treatment with ZACTRAN was efficacious for the treatment of swine respiratory clinical disease associated with virulent *App*.

Disclosure of Interest: G. Royer Conflict with: MERIAL Inc., A. Richard-Mazet Conflict with: Merial S.A.S., P. Dorr Conflict with: MERIAL Inc., O. Merdy Conflict with: Merial S.A.S., F. Joisel Conflict with: Merial S.A.S., T. Leard Conflict with: Merial Inc.

Keywords: Actinobacillus pleuropneumoniae, gamythromycin, Swine Respiratory Disease

Poster Abstracts

Bacteriology and Bacterial Diseases
ACTINOBACILLUS

PO-PF3-187

Genes with major effects on clinical, pathological and microbiological outcome of porcine pleuropneumonia

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Introduction: *Actinobacillus pleuropneumoniae* (*A. pleuropneumoniae*) is among the most important pathogens in pork production. The pathogen can cause severe economic losses due to acute or chronic pleuropneumonia accompanied by decreased performance and increased mortality. Both, therapeutic treatment and vaccination have no sustainable effect on the control or spreading of infection and in addition, vaccines are not always available. Thus, our research targets the identification of genetic markers for the resistance/susceptibility to pleuropneumonia. Such markers may be used in classical or genomic selection for *A. pleuropneumoniae* control, sustainable improvement of porcine health, production efficacy, animal welfare and consumer protection.
Materials and Methods: For achieving this aim, populations with high or low susceptible pigs based upon genetics, shortest possible linkage groups and an accurate and repeatable technology for a precise challenging and phenotyping of pigs are the major requirements. Therefore *A. pleuropneumoniae* serotype 7 was used in a standardized aerosol infection model and genetics were analysed by microarray-based differential expression studies and QTL analysis.
Results: Seven days post infection significant differences were found within different pig populations but also between litters (clinical scores 0.3 vs. 4.3). Resistance/susceptibility segregated within Large White, Landrace and Pietrain breeding lines, with highest resistance in Hampshire and highest susceptibility in Pietrain and Landrace pigs. Genes and pathways central for the defence and pathogenesis of pleuropneumonia were identified in expression analysis and gene effects, each of them explaining up to 20% of phenotypic variance, were mapped to chromosomes 2 and 12. A hotspot for gene regulation of *A. pleuropneumoniae* defence was mapped to chromosome 13. Two of the identified markers in combination explained 100% of deaths and 90% of enhanced clinical scores.
Conclusion: Populations with short linkage groups (commercial breeds), segregating for genetic resistance/susceptibility to *A. pleuropneumoniae* do exist. Identified singular genes explaining up to 20% of variance could therefore be used in genetic health selection programs. However, so far markers are not mapped as precisely as necessary for implementation in practical selection and functional genes and variants still await identification. A next generation sequencing experiment on selected pigs differing extremely in *A. pleuropneumoniae* susceptibility has been started to detect these pending basic functional gene variants.

Disclosure of Interest: None Declared

Keywords: animal welfare, consumer protection, disease resistance

Bacteriology and Bacterial Diseases
ACTINOBACILLUS

PO-PC02-001

Efficacy of one-shot Marbofloxacin treatment on development of porcine pleuropneumonia

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Introduction:
Actinobacillus (A.) pleuropneumoniae is one of the main important respiratory tract pathogens in pork production. Because of no or limited cross protection between different serotypes the disease control by vaccination is mostly hampered. In consequence high levels of antibiotics are used for combating outbreaks and limit spreading of disease. As the responsible use of antibiotics is increasingly under official control, this study aimed to investigate the efficacy of a high dose one-shot treatment with a concentration-dependent antibiotic on disease progression and success of therapy.
Materials and Methods:
For this study 36 pigs, aged 8 weeks, were infected in a standardized aerosol infection model. *A. pleuropneumoniae* serotype 2 challenge strain was used. All pigs entering the study were considered healthy by clinical and serological examination (Apx-II-ELISA). After development of clinical symptoms they were randomly divided into three treatment groups (T1: 8.0mg/kg bodyweight Marbofloxacin; T2: 2.5mg/kg bodyweight Enrofloxacin/day; T3: Saline 1ml/20kg bodyweight/day). Group T1 was treated once with active compound and with saline on the 2 following days; groups T2 and T3 were treated on three consecutive days. Afterwards the success of therapy was blindly assessed regarding lung lesions, bacteriological and clinical cure.
Results:
Eight pigs of group T3 and one of group T1 were removed due to severity of disease. There were no significant differences between T1 and T2 regarding bacteriological cure and extent of lung lesions on day 6 after infection; both were superior as compared to group T3 ($P < 0.0001$). Six days after infection there were significant differences between group T1 and T2 and group T1 and T3 regarding clinical cure and course of disease. In group T1 and T2 respiratory symptoms returned to normal within 24 hours and 48 hours after treatment, respectively ($P = 0.01$). On day 6 after infection 90.9% of the pigs of group T1, 83.3% of group T2 and 9.1% of group T3 were considered to be clinically healthy (T1:T3 = $P < 0.001$; T2:T3 = $P < 0.001$).

Conclusion:
This study confirms that a single injection of 8mg/kg bodyweight Marbofloxacin was as efficacious as a three day treatment with a licensed reference product and superior to a negative control group based on clinical and bacteriological cure. Therefore the one shot treatment with 8 mg/kg Marbofloxacin is an excellent alternative for the treatment of porcine pleuropneumonia.

Disclosure of Interest: None Declared

Keywords: Actinobacillus pleuropneumoniae, Marbofloxacin, Pleuropneumonia

Bacteriology and Bacterial Diseases
ACTINOBACILLUS

PO-PF3-052

Evaluation of meat inspection data to estimate prevalence of pleuropneumonia in Denmark

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Introduction: Patho-anatomical lesions are routinely recorded at meat inspection of swine carcasses in Denmark. The original purpose was to safeguard food. For an additional fee, a more thorough post mortem examination of lungs can be performed at the Danish Pig Research Centre (DPRC) with the specific purpose of diagnosing respiratory disorders. However, for the herd manager or veterinarian, it is tempting to use the frequent and free-of-charge recordings from the meat inspection as a proxy for post mortem examination. A previous study found weak to moderate correlations for pericarditis (R^2=0.16) and pleuritis (R^2=0.67), when slaughter house recordings were compared to post mortem examinations.
The purpose of this study was to evaluate if meat inspection recordings of *Actinobacillus pleuropneumoniae (App)*-like lesions are suitable for monitoring late outbreaks in chronically infected herds. Specifically, the objective was to estimate the correlation between the prevalence of pleuropneumonia found at post mortem examination and the prevalence of *App*-like lung lesions found at meat inspection in pigs from the same herd and batch.
Materials and Methods: From 165 herds, around 30 sets of lungs were collected at slaughter, and post mortem examination was performed at DPRC. The prevalence of chronic pleuropneumonia was considered an indicator of the within-herd prevalence of pigs undergoing a late *App*-like infection.
The meat inspection recording 'chronic pneumonia or lung abscesses (aerogenic)' was considered the equivalent to chronic pleuropneumonia and an estimate of the prevalence of *App*-like infections found at meat inspection. Hence, the prevalence of 'chronic pneumonia or lung abscesses (aerogenic)' was extracted for the entire batch of pigs from which the lung sets originated. Then, the Spearman correlation coefficient between the two prevalence measures was estimated.
Results: The median herd prevalence of pleuropneumonia found at DPRC was 3.3% (Q1-Q3: 0.0-9.1), whereas the corresponding median herd prevalence recorded at meat inspection was 0.0% (Q1-Q3: 0.0-0.6). The difference was highly significant (p<0.0001). A significant (p=0.013) Spearman´s correlation coefficient of 0.19 was estimated.
Conclusion: The prevalence of chronic pleuropneumonia was significantly higher at the post mortem examinations compared to the meat inspection recordings. Furthermore, the correlation coefficient between the two was low. Relying on routine meat inspection data for monitoring *App*-like infections would most likely result in an under-estimation of the occurrence of late *App*-outbreaks in a herd and can therefore not be recommended for diagnostic purposes.

Disclosure of Interest: G. Blach Nielsen Conflict with: Industrial PhD at MSD Animal Health, S. Saxmose Nielsen: None Declared, P. Astrup Conflict with: MSD Animal Health, J. Haugegaard Conflict with: MSD Animal Health

Keywords: Actinobacillus pleuropneumoniae, post mortem examination, slaughter house recordings

Bacteriology and Bacterial Diseases
ACTINOBACILLUS

PO-PF3-116

Vaccination against pleuropneumonia – a field trial with two different vaccines in one herd infected with Actinobacillus pleuropneumoniae serotype 2

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Introduction: Pleuropneumonia caused by *Actinobacillus pleuropneumoniae* (Ap) results in significant losses in the pig industry worldwide. Besides acute outbreaks of respiratory disease, deaths, increased antibiotic usage, condemned carcasses and lower animal welfare, there are also subclinical manifestations in an infected herd. The consequences may be a lower average daily weight gain (ADG), a higher feed conversion rate (FCR) and a high level of chronic pleurisy.
The aim of this study was to evaluate the effect of vaccination against Ap serotype 2 on FCR and the potential economic benefit of using vaccination to control both the subclinical and clinical manifestations of the disease.
Materials and Methods: In a 500-sow full-line production herd with a history of Ap-related clinical symptoms and mortality among finishers, a high FCR and a high level of chronic pleurisy, pigs were vaccinated at the age of ten to eleven weeks on arrival at the finisher sections and again three to four weeks later with either Porcilis ® APP or Hyobac APP2. The respective groups, including a control group of non-vaccinated pigs, were randomly separated into pens of 40 pigs sharing the same feed pipe. The number of pens included in the trial was 24 for each vaccine and 36 for the control group. Control pigs were bled twice (three weeks after arrival, and shortly before slaughter) to assess the serological status of Ap 2 in the batch (section). Throughout the finisher period, the use of antibiotics, number of deaths, ADG and FCR were recorded. At slaughter, a subset of lungs were sampled and sent to the Laboratory for Swine diseases in Kjellerup to investigate the prevalence and extent of Ap-related lung lesions in each group
Results: No differences were seen between the two vaccine groups and the control group in FCR, ADG, mortality, antibiotic treatments or lung lesions. During the study, no treatments for respiratory diseases were performed, and the mortality was lower than before. Furthermore, in some of the batches, the time of seroconversion occured earlier. Therefore, the vaccination time might have been too late.
Conclusion: In this study, we found no effect of vaccination against Ap, probably because the vaccination was performed too late to achieve maximum protection from the vaccines

Disclosure of Interest: None Declared

Keywords: Finisher, Pleuropneumonia, Vaccination

Poster Abstracts

Bacteriology and Bacterial Diseases
ACTINOBACILLUS

PO-PF3-255

Development of recombinant apxIA vaccine using peptide transduction domain

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Introduction: *Actinobacillus pleuropneumoniae(APP)* is an important pig pathogen, which is responsible for swine pleuropneumonia, a high contagious respiratory infection. The Apx toxins are species specific and all field strains produce these toxins. Apx toxin is consist of virulence domain A subunit and cell binding domain B subunit.
Protein transduction domains (PTD) are small peptides able to carry proteins, peptides, nucleic acid, and nanoparticles, including viral particles, across the cellular membranes into cells.
We have constructed an expression system capable of connecting the peptide transduction domain (PTD) which is known to pass through the cell and ApxIA B subunit gene. The aim of this study was to development nasal spray vaccine to more effectively induce mucosal immunity.
Materials and Methods: Bacterial strains and vectors. PTD-ApxIA B subunit cloned with pET30a vector. E. coli BL21 was used as host for transformation and expression of the recombinant protein.
Expression. Proteins expressed in E. coli were analyzed by SDS-PAGE and Western blot using mono-specific polyclonal antibody against Apx IA.
Immunization and sample collection. The group 1 of mice was inoculated by IM route. Another Groups 2-4 were inoculated by nasal route. Group 1(n=10) was inoculated with 20ug recombinant Apx1A vaccine. Group 2 (n=10), 3(n=10), 4(n=10) were inoculated with 5, 10, 20 ug recombinant Apx1A vaccine. Group 5 (n=10) injected with PBS as controls. Blood was taken on 0 and 14 days after vaccination.
Immune response analysis. Antibody titers (IgA and IgG) against recombinant ApxIA were measured by ELISA in order to analyze the immune response in the mice. The ELISA was perfomed using a mouse IgG ELISA kit and a mouse IgA ELISA kit (komabiotech) as directed by manufacturer.
Results: The expressed PTD-Apx IA B subunit proteins were shown to be 60kDa, respectively, by Western blot.
Serum IgG titers against antigen were increased compared to those of control group until the end of the study ($p < 0.05$). All mice were challenged with the mixture of the challenge strains at 3 WPPI. Among groups 1 and 3 mice, 2 and 1 were dead within 14 days after challenge, respectively. The challenge strains were isolated from lung swab of 2 mice with pneumonic lung lesions in gross examination. All mice of groups 2,4,5, however, the lungs were normal in gross examination.
Conclusion:
Suceecssfully confirmed cloning and expression of PTD-ApxI B subunit. Recombinant ApxIA genes could be a promising nasal spray vaccine candidate for the prevention of pleuropneumoniae acute infection in pigs and may provide optimal protection at target mucosal sites.

Disclosure of Interest: None Declared

Keywords: Actinobacillus pleuropneumoniae, Recombinant proteins; Immunization; Porcine pleuropneumonia; Vaccination

Bacteriology and Bacterial Diseases
ACTINOBACILLUS

PO-PF3-286

Optimization of Vaccine Production Processes of Actinobacillus pleuropneumoniae serotype2

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Introduction: *Actinobacillus pleuropneumoniae*(APP) is the etiologic agent of porcine pleuropneumonia, a worldwide endemic and highly contagious disease with high economic impact. There are 2 biovars and 15 different known serotypes. APP that require nicotinamide adenine dinucleotide (NAD) for growth are designated as biovar 1 while APP isolates that are NAD independent are designated as biovar 2. In this study, the fed-batch fermentation process was optimized for mass-production of APP serotype2 vaccine.
Materials and Methods:
-culture media
The initial culture was grown on TSB agar medium containing 50 ug/ml NAD. The seed culture was prepared in TSB broth containing 50 ug/ml NAD. The production culture medium composition was 10 g/L sucrose, 5 g/L yeast extract, 13.5 g/L potassium phosphate monobasic, 1.4 g/L magnesium sulfate heptahydrate, 50 ug/ml NAD, pH 7.0.
-fermentation condition
Batch culture was carried out in 5 L jar fermenter containing 3.6 L of production medium. The pH was controlled at 7.0 by automatic addition of 5 N NaOH or 1 N HCl. The temperature was maintained at 37 ℃. Dissolved oxygen (DO) was controlled at 20 %. The aeration rate was 1.0 vvm.
-analytical methods
The optical density was measured at 600 nm using a spectrophotometer. Cell concentration was determined by measuring dry cell weight (DCW). The concentration of carbon source was analyzed according to the 3,5-dinitrosalicylic acid (DNS) method.
Results:
-effect of carbon source
Various carbon sources were used to glucose, galactose, sucrose, fructose and lactose for carbon source optimization. Sucrose as carbon source showed high cell mass production. Culture sample of used to sucrose was 2.8 g dcw/L.
-cell production of batch fermentation
The batch fermentation was completed in 5 hours with a final cell mass of 4.7 g dcw/L. The residual sucrose concentration rapidly fell down after 2 hours of fermentation due to the initiation of cell growth. The residual sucrose concentration went down to 0.6 g/L after 5 hours.
-optimization of fed-batch fermentation
The feeding solution composition was 400 g/L sucrose, 130 g/L yeast, 50 ug/ml NAD. Feeding was started at 3 hours in response to sucrose level dropping to 5 g/L. The feeding pump controlled the feed rate to maintain DO level at 20 % air saturation. The maximum cell density reached to 10.4 g dcw/L in 8 hours. The cell density was higher than batch fermentation.
Conclusion: Fermentation conditions were optimized to increase the concentration of cell mass. Mass production through optimized fed-batch fermentation process could provide the basic for the industrial production of APP2 vaccine.

Disclosure of Interest: None Declared

Keywords: Acinobacillus pleuropneumoniae serotype2, Fed-batch culture, Fermentation

Bacteriology and Bacterial Diseases
ACTINOBACILLUS

PO-PF3-125

Serotypes of Actinobacillus pleuropneumoniae detected in Chile during the period 2013-2015

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Introduction: Actinobacillus pleuropneumoniae (App) is the causative agent of Swine Pleuropneumoniae. Among the most important virulence factors that App possesses are the following toxins: apxI, apxII, apxIII and apxIV. Today, there are 15 serotypes and the toxins found in each of them could be one or their combinations of these and they have been detected with different prevalence depending on the geographical location in the world. Vaccination has been an important control factor in swine herds; however, it is of great importance to know App serotypes in farms in order to reach optimal prevention and immunogenicity of a biological product used to control it.
The aim of this study was to identify App serotypes detected in Chile during outbreaks occurred between 2013 and 2015.
Materials and Methods: Between 2013 and 2015, at least 20 different App acute outbreaks occurred in pig farms in Chile. Sample of lungs with typical App lesions coming from 25% of the industrial swine farms in Chile were processed in Virbac-Centrovet. From each lung sample, a swab was taken and plated on chocolate agar. Each chocolate agar plate was incubated in microaerobic environment at 37°C for 24 hours. From isolated colonies and following the protocol described by Schaller et al., 2001, total DNA was extracted for PCR identification of the App. Each positive App colony was subsequently cultivated under anaerobic conditions with 5% of CO2 in liquid BHI + 1%Vitox for 20 hours at 37°C. Each culture was centrifuged, from the bacterial pellet a DNA extraction and then it was quantified by spectrophotometry. The identification of the serotype of App using primers that amplify specific regions of the APXIA, APXIB, APXII, APXIII, APXIV genes, according to the protocol described by Rayamajhi et al, 2005.
Results: From the isolated colonies, App serotypes 1 - 4 - 6 and 8 were identified by using PCR. The serotype that was found in greater frequency and affected a greater number of the assessed farms corresponds to serotype 4(40%). Whereas serotype 1, 6 and 8 were detected in one farm each.
Conclusion: In recent years, the frequency of cases of App has increased in the domestic industry resulting in important economic and production losses. The diversity of App serotypes detected in Chile, has had a negative impact on the effectiveness of traditional vaccines. Thus, during the last years, the use of autogenous vaccines for the prevention and control of swine pleuropneumoniae has increased.
Based on the cases / farms analyzed, no more than one serotype was found in any of them. It will be necessary to continue to monitor the farms in the following years in order to evaluate potential new serotypes not detected in this study.

Disclosure of Interest: None Declared

Keywords: Actinobacillus pleuropneumoniae, serotype , Chile

Bacteriology and Bacterial Diseases
ANTIMICROBIAL

PO-PF3-185

Pen-level agreement of antimicrobial resistance from pooled faecal samples at pen level and diarrhoeic pigs

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Introduction: Antimicrobial resistance testing of enterotoxic *Escherichia coli* (*ETEC*) is used as a diagnostic decision tool for selecting classes of antibiotics for treatment in pigs. Resistance testing is often done on isolates from faecal samples collected from diseased case pigs with the advantage of high certainty of analysing *E. coli* stains that has caused disease in the individual pig. The objective of this current study was to investigate pen-level agreement of antimicrobial resistance testing of *E. coli* isolates sampled from diarrhoeic pigs and from pooled faecal pen floor samples (PFP).
Materials and Methods: Faecal samples from diarrhoeic pigs two to four week post weaning and from PFP were collected from three commercial nursery facilities in the eastern part of Denmark. The samples were cultured on blood agar and *E. coli* isolates were analysed by PCR for adhesion factor, and toxin genes. Isolates possessing genes for both adhesion factors and toxins were classified as virulent and resistance testing by Sensititre for Tetracycline (TET), MIC=16 µg/ml; Ampicilline (AMP), MIC=32 µg/ml; Sulphamethoxazole (SUL), MIC=512 µg/ml; Trimethoprim (TMP), MIC=16 µg/ml; Streptomycin (STREP), MIC=32 µg/ml; and Spectinomycin (SPEC), MIC=128 µg/ml was performed. In the data analysis a pen was classified as antibiotic resistance positive against a specific antimicrobial compound if one virulent *E. coli* strain from minimum one pig in the pen were resistant. Similar a PFP sample were classified as antibiotic resistance positive if one virulent *E. coli* strain from the sample were resistant. Agreement was calculated by comparing the pen classification with the results from the PFP sample.
Results: A total of 89 virulent *E. coli* isolates were cultures from 22 (26%) of 86 sampled diarrheic pigs and from in 13 (41%) of 31 pooled faecal samples from the pen floor. The overall prevalence of antimicrobial resistance in isolates for TET, AMP, SUL, TMP, STREP and SPEC was; 47.2 %, 60.7 %, 69.7 %, 69.7 %, 34.8 % and 18.0 %. It was possible to compare pen-level results from pig faecal samples and pen floor samples in 10 pens. Complete agreement was obtained for all antibiotic classes except for STREP where agreement was found in 8 of 10 pens.
Conclusion: The agreement of antimicrobial resistance testing of virulent *E. coli* strains was high between PFP samples and pigs samples. This study indicates that antimicrobial resistance testing can be performed on pooled faecal pen samples, as a tool for selecting classes of antibiotics for treatment of pigs at the pen level. However, these results should be confirmed in a larger study.

Disclosure of Interest: None Declared

Keywords: antimicrobial resistance, diarrhoea, nursery pigs

Poster Abstracts

Bacteriology and Bacterial Diseases
ANTIMICROBIAL

PO-PF3-073

Therapeutic efficacy of ZACTRAN® (gamithromycin) against Swine Respiratory Disease in a multi-center field trial in Europe

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Introduction: Respiratory infections remain a significant problem for the swine industry and result in substantial economic loss. Swine Respiratory Disease (SRD) is a complex condition involving viral agents and bacterial agents. ZACTRAN is a novel azalide antibiotic (gamithromycin) currently licensed in Europe, North America and other regions for the treatment and control of bovine respiratory disease and recently licensed in Mexico and Brazil for the treatment of swine respiratory disease (SRD) associated with A. pleuropneumoniae, H. parasuis, B. bronchiseptica and P. multocida.
Materials and Methods: From a total of 6 sites located in 3 different EU countries, 305 growing pigs displaying clinical signs of SRD were randomly assigned to receive a single intramuscular dose of ZACTRAN at 6.0 mg/kg body weight (BW) or Zuprevo® (MSD AH) at 4.0 mg/kg BW. Three sentinels animals at each site meeting the inclusion criteria were euthanized and necropsied prior to treatment to obtain samples for detection of pathogens and site qualification.Each enrolled animal was clinically assessed daily for clinical signs including depression, respiratory scores, and rectal temperature. Over the course of the study (from Day 0 to Day 10), pigs meeting SRD removal criteria or non-SRD concurrent pathological conditions were removed from the study. Nasal swabs or bronchoalveolar lavages (BALs) were collected for each enrolled animal on Day 0 prior to treatment for bacterial culture and SRD target pathogen identification.General and local tolerance was also assessed. On Day 10, all remaining animals were evaluated as treatment success or failure. The percentage of success and each clinical parameter were compared using a non-inferiority hypothesis test.
Results: The presence of the 4 pathogens in association with SRD clinical signs was confirmed for the 6 sites from swabs or BALs. From Day 2 to Day 8, 8 animals (3 in ZACTRAN group and 5 in Zuprevo group) were removed from the study displaying SRD removal criteria. On Day 10, the proportion of treatment success was 97% in ZACTRAN and 93% in Zuprevo, ZACTRAN being equivalent to or better than Zuprevo. Following injection, only minor local reactions (eg. redness) were observed in six out of the 153 animals treated with ZACTRAN and 10 out of the 152 animals treated with Zuprevo. These reactions were very limited and resolved rapidly within one day.
Conclusion: Under the conditions of this trial, a single intramuscular injection of ZACTRAN administered to swine at 6 mg/kg provided clinical cure of 97% of pigs with spontaneously acquired SRD and was shown to be statistically equal or better than a reference product approved for the treatment of SRD.

Disclosure of Interest: A. Richard-Mazet Conflict with: Merial S.A.S., H. Dietmar Conflict with: Merial GmbH, F. Voisin: None Declared, I. Bohne: None Declared, F. Fraisse Conflict with: Merial S.A.S., R. Winter Conflict with: Merial GmbH, P. Dumont Conflict with: Merial S.A.S., P. Jeannin Conflict with: Merial S.A.S.

Keywords: efficacy, gamythromycin

Bacteriology and Bacterial Diseases
ANTIMICROBIAL

PO-PF3-241

Therapeutic efficacy of ZACTRAN® (gamithromycin injectable solution) against Swine Respiratory Disease in a field trial in Japan

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Introduction: Swine Respiratory Disease (SRD) is a complex condition involving bacterial agents such as *A. pleuropneumoniae (App)*, *H. parasuis* and *P. multocida (Pm)*. These organisms often act together to increase the severity and duration of the disease. Appropriate control of these pathogens is important for swine health management. This study aimed to compare the field efficacy of ZACTRAN®, Merial in comparison with Danofloxacin mesylate (ADVOCIN®, Zoetis) for the treatment of naturally occurring SRD under field conditions.
Materials and Methods: The study was conducted on 2 farms, where pigs are known to have SRD associated with *App* and *Pm*. Each site was recruited based on the confirmation of SRD clinical signs associated with necropsy lesions and successful isolation of these target pathogens in lung tissue in 5 sentinel pigs per site.
Sixty three 2-to-3 month-old SRD-unvaccinated pigs that had not been vaccinated against SRD or received medication that would have impacted treatment response were recruited on Day 0 (D0). On each test site, pigs from the same batch were included by assessing clinical signs of SRD using clinical scores for respiratory condition, cough, physical activity, appetite and recording rectal temperature. Pigs having a minimum level of a composite clinical score and a body temperature of at least 39.5°C were enrolled in the study. Allocation to the treatment groups was performed randomly in replicates of 3 animals in a 2:1 ratio of ZACTRAN-treated pigs (6 mg/kg) to ADVOCIN-treated pigs. On D0, ZACTRAN was administered intramuscularly at 6.0 mg/kg bodyweight (BW) once; ADVOCIN was injected once daily for 3 consecutive days intramuscularly at 1.25 mg/kg BW or 2.5 mg/kg BW depending on the level of the composite clinical score.
Pigs were clinically monitored daily for their clinical scores until D14. A clinical improvement index was calculated for each pig using the clinical scores recorded on D0 and D7. The proportion of improved pigs for each treatment was compared using a non-inferiority hypothesis test (non-inf. margin = 0.15).
Results: No health abnormality other than SRD related symptoms was observed in any animals treated with ZACTRAN. No local reactions were observed in any ZACTRAN-treated animals. The proportion of animals judged to have improved clinical index was 83% in the ZACTRAN single-treated group and 71% in the ADVOCIN 3-times-treated group. This result supported that the efficacy of ZACTRAN was equivalent to or better than ADVOCIN.
Conclusion: Under the conditions of this trial, a single intramuscular injection of ZACTRAN administered at 6 mg/kg to pigs was shown to be effective for the first line treatment of spontaneously acquired SRD by providing clinical cure of 83%.

Disclosure of Interest: Y. Kondo Conflict with: Merial Japan Limited, N. Nakanishi: None Declared, Y. Wakui Conflict with: Merial Japan Limited, A. Richard-Mazet Conflict with: Merial S.A.S., G. Kinoshita Conflict with: MERIAL S.A.S., P. Jeannin Conflict with: Merial S.A.S.

Keywords: efficacy, first-line treatment, gamythromycin

Bacteriology and Bacterial Diseases
ANTIMICROBIAL

PO-PF3-046

Monitoring antimicrobial consumptions in fattening pigs in Italy: preliminary findings towards an integrated approach

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Introduction: Italy is a large pig producer and a system to monitor active ingredients (AIs) consumptions of veterinary medicinal products (VMPs) at farm level is needed. The aims of this study were to develop a tool to record these consumptions and to compare AIs usages with production losses, biosecurity levels and health statuses.
Materials and Methods: A data collection software, an XML database and an interactive dashboard were developed to store data and perform calculations. The system was tested with a convenience sample of 20 fattening farms (mean pig slaughtered per year 4780). Data were collected retrospectively for 2014 or 2013.
AIs consumptions were calculated yearly as milligrams of AI used per kilogram of meat produced (mg / kg meat). In addition, defined daily and course dose animal for Italy (DDDAit and DCDAit), based on national prescriptions, were established. Mean days and courses of therapy per pig were also calculated using 100 kg as average weight at treatment.
Biosecurity levels were evaluated with a survey and losses as sum of mortality and cull. Correlations between AIs usages and losses or biosecurity were investigated. To further assess differences in AIs consumptions, farms were grouped according to clinical reports, presence or absence of *Brachyspira hyodysenteriae* and *Actinobacillus pleuropneumoniae* (APP).
Results: Average usages were 114 mg / kg meat (range; 20–222), 17.7 days (range; 4.1–37.9) and 3.3 cycles (range; 0.6–6.9) per pig. Administration routes were 4.1% injectable (of total DDDAit), 22.6% oral powder, 10.9% oral solution and 62.4% premix. The top five used AIs were lincomycin (20.4% of consumed DDDAit), doxycycline (16.5%), tiamulin (15.6%), amoxicillin (12.9%) and colistin (10.1%). Mean biosecurity score was 63.0% (range; 48.9%–73.9%). Mean losses were 5.2% (range; 2.0%–10.0%). AIs consumption and biosecurity or losses were not significantly correlated. 35% of the farm reported respiratory signs, 20% enteric and 45% both. 30% were positive to *B. hyodysenteriae*, 25% to APP, 10% to both and 35% were negative. AIs consumptions did not significantly differ between groups.
Conclusion: An XML database allows changing bases of calculation, when new standards are established, without affecting stored data. Interactive dashboards offer an intuitive depiction of AIs consumptions, via charts, with different levels of aggregation.
Evaluations on national consumptions and comparisons between AIs usages, losses, biosecurity and health status require further studies with a larger sample size. Data on animal welfare, slaughterhouse and other pathogens should be included to improve the health status assessment and the integrated approach.

Disclosure of Interest: None Declared

Keywords: Antimicrobials consumption, DDD, fattening pigs

Bacteriology and Bacterial Diseases
ANTIMICROBIAL

PO-PF3-036

Florfenicol susceptibility survey of EU field isolates of Actinobacillus pleuropneumoniae, Haemophilus parasuis and Pasteurella multocida.

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Introduction: Florfenicol is a well recognized, broad spectrum antimicrobial with a high lipophilicity resulting in fast, significant serum concentrations and very good tissue distribution. Oral administration leads to an almost complete absorption of the molecule. Amphen® 200 mg/ g oral granules is a patented formulation containing 200 mg/ g florfenicol for use in drinking water of pigs. In this study the florfenicol susceptibility of EU field isolates of 3 major swine bacterial pathogens was tested.
Materials and Methods: Susceptibility testing was carried out in a fully Good Laboratory Practice compliant study in one central laboratory. All tests were in accordance with the procedures of the Clinical and Laboratory Standards Institute. 222 Strains of bacterial pathogens from cases of porcine respiratory infections were collected in 7 European countries over the period 2008 - 2010. Representative strains of *A. pleuropneumoniae* (n=67), *H. parasuis* (n=54) and *P. multocida* (n=101) were tested in this large scale study. The MIC90 value (Minimal Inhibitory Concentration) for a specific bacteria is defined as the lowest concentration of an antimicrobial to inhibit growth of 90 % of the bacterial strains in vitro.
Results: In this survey the MIC ranges for *A. pleuropneumoniae, H. parasuis* and *P. multocida* were respectively 0.06 - 1.0 µg/ ml, ≤0.015 - 1 µg/ ml and 0.25 - 1 µg/ ml with a very tight MIC distribution. The florfenicol MIC90 value for all three pathogens was determined at 0.5 µg/ ml and far below the clinical breakpoints (for *A. pleuropneumoniae* and *P. multocida* : susceptible ≤ 2 µg/ ml and resistant ≥ 8 µg/ ml). In comparison with different elaborate studies over the last 15 years, no changes in MIC data were noticed.
Conclusion: MIC values of florfenicol for three major swine respiratory pathogens are very favorable and do not seem to change over time. This confirms reports about the efficacy of the molecule in the field. The main hurdle for in water application was the absence of a practical water soluble florfenicol. The innovative formulation of Amphen® 200 mg/ g for use in bulk tanks and proportioners ensures a high success rate.

Disclosure of Interest: None Declared

Keywords: Amphen®, Florfenicol, Susceptibility

Poster Abstracts

Bacteriology and Bacterial Diseases
ANTIMICROBIAL

PO-PF3-223

Efficacy of Amphen® 200 mg/ g against porcine respiratory infections : a case report

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Introduction: Amphen® 200 mg/ g is a new formulation of florfenicol for use in drinking water of pigs. Its efficacy against *A. pleuropneumoniae* (MIC90 value 0.5 µg/ ml) was tested in a farrowing to finishing herd of 300 sows. Despite vaccination of the piglets against PCV2 and *M. hyopneumoniae*, the fatteners suffered with chronic pneumonia. The all-in/ all-out principle was not maintained in the fattening units. Dyspnoea, coughing, nasal flow and anemia resulted in disappointing technical performances. Pigs (20-115 kg) were formerly intensively injected or orally treated with antimicrobials with a poor success. A yearly mortality rate of 5.8 % was registered and 70.1 % of this mortality was noted in the pre-fattening period (20-45 kg).
Materials and Methods: Multiple necropsies showed haemorrhagic, necrotizing pneumonia, indicating *A. pleuropneumoniae* infections. These findings were confirmed by PCR tests and culture (biotype 2, serotype 3). Slaughterhouse examination of lungs (n=145) also revealed pleuritis and abcesses in 20 % of the lungs. Susceptibility testing of the isolated *A. pleuropneumoniae* strain was performed and florfenicol MIC was determined at 0.38 µg/ ml. Taking into account the clinical breakpoint of florfenicol against *A. pleuropneumoniae* (sensitivity : ≤ 2 µg/ ml), the causative pathogen was considered as completely susceptible. In the pre-fattening unit, a treatment with Amphen® during 5 days was advised at a daily dose of 10 mg florfenicol per kg bodyweight (n=325) and the results were compared to an untreated control group (n=285). Clinical observation and mortality were the assessed parameters.
Results: Two days after the end of the treatment, pigs in the treatment group were clinically healthy. They did no longer suffer with pneumonia and nasal excretions at all. Consequently an increased feed intake was noticed. No mortality was registered anymore in the treatment group the first 3 weeks after the start of the treatment. On the contrary, pigs of the untreated control group still had a very pronounced cough and were less active. In the same period mortality was registered to be 1.7 % (n=5).
Conclusion: Amphen® 200 mg/ g is a first choice antimicrobial to control respiratory infections in pigs, based upon the clinical results and the favorable MIC values.

Disclosure of Interest: None Declared

Keywords: Actinobacillus pleuropneumoniae, Amphen®, Florfenicol

Bacteriology and Bacterial Diseases
ANTIMICROBIAL

PO-PF3-242

Evaluation of the effect of early metaphylactic use of ceftiofur on the intestinal morphology in pigs using stereological methods- a preliminary study

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Introduction: A metaphylactic use of ceftiofur during the first days of life is a very common way in Austrian swine production to prevent bacterial diseases. Little information exists whether ceftiofur affects the intestinal structure resulting in impaired gut function and development in nursery pigs. Different approaches have been used to describe the morphology of the intestinal wall quantitatively. Villus heights and crypt depths are frequently used parameters to describe physiological and pathological changes in the intestine. The aim of this preliminary study was to assess the relative mucosal surface area with stereological methods to ascertain whether antibiotics might affect the relative resorptive and secretory surface of the intestine, which segment might be affected most and if males and females respond differently.
Materials and Methods: Litters of 2 sows were included. Piglets (n=8) were randomly assigned into a treatment group (AB; n=4) or a control group (control; n=4). 12 h post partum AB piglets received an intramuscular injection of ceftiofur (5.0mg/kg), control piglets a placebo (0.2ml/kg physiological NaCl solution). Piglets were euthanized at weaning and tissue samples from the duodenum, jejunum, ileum, caecum and colon were taken. Samples were fixed in formalin. Vertical uniform random sampling for tube-like hollow organs was applied during histological processing. Histological sections were stained with periodic acid- Schiff reagent.Inner and outer surface of the intestine were assessed using a cycloidal stereological grid. The relative resorptive and secretory surface was calculated as relation between inner and outer surface. Data were subjected to ANOVA using PROC Mixed of SAS® and were analyzed as repeated measures over gut sites.
Results: As expected, the relative resorptive and secretory surface was largest in duodenum, linearly decreased until the caecum and increased again in the colon ($P < 0.001$). Preliminary results showed that the relative resorptive and secretory surfaces at all gut sites were not influenced by the ceftiofur injection early in life ($P > 0.1$). However, there was a great variation among piglets and the piglets from the two sows appeared to respond differently.
Conclusion: Preliminary results showed that ceftiofur injection may have no effect on the relative resorptive and secretory surface. However, due to the variation among pigs and litters, further analysis of the complete AB and control group will be performed. In the main experiment, the jejunum and the colon will be analysed as representative gut sites for the small and large intestine.

Disclosure of Interest: None Declared

Keywords: antibiotics, intestine, stereology

Bacteriology and Bacterial Diseases
ANTIMICROBIAL

PO-PF3-040

Comparative viscosity and syringeability of five florfenicol injectable solutions

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Introduction: Easiness of injections is an important parameter for animal welfare and operators. This study was done to compare viscosity and syringeability of 5 florfenicol injectable solutions marketed in Mexico or Brazil, under laboratory conditions.
Materials and Methods: One 40% florfenicol injectable solution (Maxflor® LA, Virbac) was compared to four 30% florfenicol injectable solutions. Viscosity was measured at ambient temperature and 5°C by using a Brookfield viscosimeter. Syringeability was measured according to two tests: either as the time necessary to extract a 10 mL volume from a product vial by applying a constant strength of 19.6 N on a syringe or as the time necessary to empty a syringe containing a 10 ml volume of product by applying the same constant strength of 19.6 N. Two temperatures (ambient one ranging from 19 to 22°C and 5°C) and 2 types of needle (16G, 18G) were tested. Each measure was repeated 6 times with a chronometer. Times were then converted to the times corresponding to a volume necessary to treat a pig weighing 80 kg for a florfenicol dose regimen of 15 mg/kg, assuming a proportionality of the time according to the volume. For syringeability tests, each 30% florfenicol solution was compared to the 40% florfenicol solution by the t test with Bonferroni correction for pairwise multiple comparisons.
Results: Viscosity ranged between 36 and 100 mPa.s at ambient temperature and between 96 and 328 mPa.s at 5°C, the 40% florfenicol solution having the lowest viscosity at both temperatures. The mean time to extract the defined volume at ambient temperature ranged from 2.2 to 7.0 s with a 16G needle and from 5.2 to 17.9 s with an 18G needle. The corresponding mean time at 5°C ranged from 5.0 to 19.9 s with a 16G needle and from 12.2 to 51.6 s with an 18G needle. The mean time to empty the syringe from the defined volume at ambient temperature ranged from 1.4 to 4.4 s with a 16G needle and from 3.3 to 10.4 s with an 18G needle. The corresponding mean time at 5°C ranged from 2.7 to 10.0 s with a 16G needle and from 6.4 to 23.2 s with an 18G needle. The 40% florfenicol solution showed the lowest times for all conditions, differences being statistically significant with all the 30% solutions.
Conclusion: Precision of the syringeability test was satisfactory. Measured times increased as viscosity of the product increased. Times were longer for the narrower gauge needle and at low temperature. Shortest times were recorded for the solution having both the highest florfenicol concentration and the lowest viscosity, reflecting influence of excipients. For this formulation, times appeared compatible with animal welfare and practical constraints (metaphylaxis, lower temperature in winter).

Disclosure of Interest: None Declared

Keywords: Florfenicol, Syringeability, Viscosity

Bacteriology and Bacterial Diseases
ANTIMICROBIAL

PO-PF3-294

Main antimicrobials used and principal reasons for their use in Irish pig farms

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Introduction: Antimicrobial resistance (AMR) is a major human and animal health problem that is linked with antimicrobial usage. Given current knowledge of the problems caused by AMR in humans and animals, it is important to explore the reasons for differing levels of antimicrobial use in Irish pig production and assess its impact on animal health and performance.
Materials and Methods: A questionnaire including 90 questions on different aspects of pig production such as health, antimicrobial use, welfare, management practices and biosecurity was designed by the research team and reviewed by policy makers and private pig veterinary practitioners. Between November and December 2014, 80 questionnaires were distributed to pig farmers at various events with provisions for return by post. Results were entered in Excel and descriptive statistics of the data produced.
Results: A total of 28 pig farmers (9% of commercial Irish herds) returned questionnaires. The average number of sows on their farms was 737 (range 83 to 2900). The number of litters per sow per year was between 2.1 and 2.4 and the number of pigs born per sow per year ranged from 26 to 34.5. The main reasons cited for the use of antimicrobials in piglets were diarrhoea (52%), lameness (16%) and poor growth (16%). In first stage weaned pigs, meningitis and poor growth were reported by 25% of farmers and diarrhoea and respiratory problems by 17%. In second stage weaned and finishing pigs the main problems reported were respiratory issues (32 and 26%), poor growth (20 and 13%) and lameness (16 and 17% respectively). Lameness (40%) and MMA (12%) were the principal reasons for antimicrobial use in sows. In-feed antimicrobials were mostly used in first (42%) and second (32%) stage weaned pigs. Piglets, finishing pigs and sows were mainly treated by injected antibiotics. A wide variety of antimicrobials were used in piglets with more than 10 products cited. In growing and finishing pigs use of between 4 and 6 different antimicrobials was recorded, with penicillins and tetracyclines being the most common.
Conclusion: The 28 farms were representative of the Irish pig industry in terms of size, performance figures and geographical location. The main reasons given for the use of antimicrobials were poor growth, respiratory disease and lameness. In-feed medication was used mainly in first and second stage weaned pigs and penicillins and tetracylines were the most commonly employed antimicrobials. This preliminary study will be extended to a higher percentage of farms during 2016.

Disclosure of Interest: None Declared

Keywords: Antimicrobials, Ireland, pig production

Poster Abstracts

Bacteriology and Bacterial Diseases
ANTIMICROBIAL

PO-PF3-277

Gut Health – A continuous challenged eco-system.

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Introduction: The widespread use of antimicrobial agents in human and veterinary medicine has favoured the spread of resistance. In view of the transmission risk of highly antibiotic-resistant strains between animals and humans, controlling resistance is essential to safeguard the future efficacy of antimicrobial agents in veterinary as well as in human medicine. The ban on antibiotic growth promotors in the EU was a first step in the strategy to deal with antibiotic resistance. Today many countries in the EU, but also outside the EU-borders, are taking initiatives to reduce medication use in general and in animal feed in particular.
Materials and Methods: A piglet trial has been set-up on a good managed farm of 5000 sows. From an health point of view, the farm reflects very well the typical Italian situation with PRRS pos., mycoplasma pos., streptococcus suis pos., PCV2 + and Aujeszky neg animals. E. Coli diarrhea after weaning is not a major issue if medicated well.
The trial was set-up with 240 piglets, 2 repetitions, 3 treatments, 2 pens/treatment and 20 piglets/pen. The piglets are weaned at 24-26 days of age and the trial took place from weaning till 20 days after weaning.
A positive control (PC) was supplemented with the standard antibiotic cocktail consisting out of 500 ppm Amoxiciline, 120 ppm Colistine and 3000 ppm Zn-oxide. As the new legislation allowed only 1 single molecule policy, a negative control (NC) was supplemented with only 500 ppm Amoxiciline. Finally, the trial group (T) contains 500 ppm Amoxiciline combined with esterified butyrates and plant extracts (1 kg/MT).
In case of digestive upset, the animals are treated individually with Enrofloxacine for 2 consecutive days.
Results: The performance data clearly demonstrated the positive effect of the antibiotic cocktail as the PC-group performed better compared to the NC-group (ADG of 206 g/day vs 156 g/day respectively). The trial group T performed equally with an average daily gain of 200 g/day.
The average number of individual treatments/trial day was 4,14 for the negative control. A significant improvement was noticed for the trial group (T) (1,55) and 0,45 for the positive control. The fecal scoring followed the same trend.
Conclusion: Well selected active ingredients like butyric acid and well-chosen botanical extracts, combined in an optimal blend have a clear potential to improve intestinal health, reduce intestinal disorders and eventually allow reduction of antibiotic growth promotors and in feed used antibiotics, while maintaining or even improving performance data.

Disclosure of Interest: None Declared

Keywords: gut health

Bacteriology and Bacterial Diseases
ANTIMICROBIAL

PO-PF3-194

Detection of Extended Spectrum Beta-Lactamases Among Enterobacteriaceae strains isolated from pigs

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Introduction: Brazil has emerged as a leading global exporter of pork, occupying the fourth position. In this regard, the high amount of antimicrobial agents used in intensive systems of animal production has been pointed as a factor of selection and persistence of resistant bacteria. Thus, the aim of this study was evaluated the presence of Extended-Spectrum Beta-lactamases-producing (ESBL) gram-negative bacteria in swine Brazilian farms.
Materials and Methods: In 2012, 400 fecal swabs collected from nursing (40 days) and finisher (90 days) pigs, male and female, from 33 farms located in seven Brazilian states were screened for ceftiofur-resistant enterobacterial strains. Firstly, the swabs were plated in MacConkey agar supplemented with ceftiofur 2 µg/mL. Next, the isolates were identified by MALDI-TOF MS and the resistance profile was determined by agar and/or microdilution method. The presence of ESBL genes was examined by PCR. Finally, the clonal relatedness was evaluated by ERIC-PCR.
Results: A total of 400 healthy animals were evaluated regarding the presence of bacterial producers of ESBL, being isolated 66 ESBL-positive strains. *Escherichia coli* grouped 51 isolates carrying bla_{ESBL} genes (CTX-M-2, CTX-M-15 and CTX-M-8), followed by four CTX-M-15 producing *Klebsiella pneumoniae* isolates. The remaining ESBL producers were *Salmonella enterica* (2), *Pseudomonas aeruginosa* (3), *Morganella morganii* (1), *Proteus mirabilis* (4) and *Enterobacter cloacae* (1). All strains isolated showed high MICs to ceftiofur (MIC≥256 mg/L), cefotaxima (MIC≥128 mg/L) and ceftriaxona (MIC≥256 mg/L). ERIC-PCR revealed the multiclonal dissemination of ESBL producers, suggesting the spread of $bla_{CTX\text{-}M\text{-}type}$-carrying plasmids. The overall prevalence of ESBL producers were 16.5% (66/400), but the Minas Gerais state showed concentered 54% of CTX-M+ strains, suggesting a local challenge in production practices. In fact, ESBL production has been widely reported in our country among community and hospital settings. Of great interest, we report the emergence of CTX-M-like encoding genes in food-producing swine and for the first time, the presence of CTX-M-15+ *Klebsiella pneumoniae* in Brazilian animal production.
Conclusion: The surveillance of antimicrobial resistant phenotypes in food-producing animals must be continuous in order to adopt measures to prevent their dissemination in the farms and their release into human population, such as the rational use of antimicrobials, administration of narrow spectrum drugs always is possible, strict sanitary measures and disinfection of farms with detected presence of ESBL producers.

Disclosure of Interest: None Declared

Keywords: E. coli, ESBL, Resistance

Bacteriology and Bacterial Diseases
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Antimicrobial resistance of pathogenic NAD-dependent Pasteurellaceae from pigs

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Introduction: *Actinobacillus pleuropneumoniae* (*App*) and *Haemophilus parasuis* (*Hps*) are fastidious bacterial species commonly isolated in clinical specimens from pigs. *App* is associated with necrotizing pneumonia and *Hps* with polyserositis, arthritis and meningitis in pigs. The treatment has traditionally involved using beta-lactam antibiotics, to which the isolates of both pathogens have been almost universally susceptible. However, high frequencies of resistance to several groups of antibiotics, including beta-lactams, have emerged in some other countries, so currently the treatment has to be based on local knowledge. The aim of the study was to assess the prevalence of antimicrobial resistance of both *App* and *Hps* isolated from pigs in Poland.
Materials and Methods: A total of 133 *App* and 67 *Hps* strains isolated, from diseased pigs, between 2008 and 2015 were tested. A single isolate from the same farm was included. The strains were analyzed for their resistance to 11 antimicrobials using the microbroth dilution method (TREK D. S.). The obtained MIC (minimal inhibitory concentration) values were evaluated according to the criteria CLSI (clinical breakpoints, 2013).
Results: Resistance of *App* isolates was found against 9 tested antimicrobials, reaching the highest values for tetracycline (30%), ampicillin and penicillin (6% each), erythromycin (4.5%), enrofloxacin (2.3%), tiamulin (1.5%), trimethoprim/sulphamethoxazole, spectinomycin and ceftiofur (0.8% and one strain, each). The significant proportion – 90% and 60 % isolates – had a reduced susceptibility to erythromycin and tetracycline, respectively. No resistance against amoxicillin/clavulanic acid and florfenicol was detected.
Resistance of *Hps* isolates was found against 8 tested antimicrobials, reaching the highest values for tetracycline (17.1%), enrofloxacin (13.2%), trimethoprim/sulphamethoxazole, ampicillin and penicillin (9.2% each), tiamulin and erythromycin (2.6% each). The significant proportion (90%) isolates had a reduced susceptibility to erythromycin only. No resistance against amoxicillin/clavulanic acid, ceftiofur and florfenicol was detected.
Conclusion: This study showed that Polish *App* and *Hps* strains had similar trends of the resistance. So far the isolates of *App* and *Hps* have full susceptibility to amoxicillin with clavulanic acid as well as florfenicol. *App* isolates have very limited degree of resistance to ceftiofur. The pathogenic NAD-dependent *Pasteurellaceae* have a limited to moderate degree of resistance to natural and semisynthetic penicillins, which are commonly used for treatment. Our results also showed a higher level of resistance to enrofloxacin and sulfamethoxazole/*trimethoprim* combination in *Hps*, than *App* strains.

Disclosure of Interest: None Declared

Keywords: Actinobacillus pleuropneumoniae, Haemophilus parasuis, MIC

Bacteriology and Bacterial Diseases
ANTIMICROBIAL

PO-PF3-140

The risk of cross-contamination due to the use of antimicrobial medicated feed throughout the trail of feed from the feed mill to the farm

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Introduction: The cross-contamination of non-medicated feed with residues of antimicrobials causes a public and animal health concern associated with the potential for selection and dissemination of resistance in commensal and potentially zoonotic bacteria. To identify the extent of the situation, we built a model that provides a way to estimate the percentage of cross-contaminated pig feed in total and per different levels at which cross-contamination may occur (i.e. the feed mill, the transport truck, the farm), for different levels of antimicrobial medicated feed (AB MF) produced in a country per year.
Materials and Methods: The potential carry-over of AB traces was assessed from the feed mill to the transport truck and the storage and distribution at the farm. The model was thus subdivided in three modules, one for each level, and five exposure pathways were considered. It was built using @Risk® software (Palisade Corporation®) and was run at 10,000 iterations per simulation.
Results: The model estimated that, given our assumptions, when a hypothetical level of x_i=2% of the feed produced in a country per year is AB MF, C_{1i}= 5.5% (95%CI 3.4%; 11.4%) of the total feed produced in a year (T_{1i}) could be cross-contaminated with different levels of AB due to practices related to MF. In detail, 1.80% (95%CI 0.2%; 7.7%) of T_{1i} in such a country would be due to cross-contamination occurring at the feed mill, 1.83% (95%CI 1.3%; 2.0%) at the transport truck and 1.84% (95%CI 1.2%; 2.0%) at the farm level. The model also demonstrated that, even in cases where MF would be produced in end-of-line mixers or fine dosing system (FDS) trucks would be used, the risk would not be completely removed; the percentage of cross-contaminated feed produced in a country (where x_i=2%) per year would be C_{2i}=3.7% (95%CI 2.9%; 4.0%) and C_{3i}=2.4% (95%CI 1.6%; 2.7%), respectively.
Conclusion: The model showed a real risk of cross-contamination of feed due to practices related to MF. The risk is hard to be fully removed and thus, the use of MF should be avoided as much as possible. The model demonstrated that a considerable risk of cross-contamination can be avoided when MF is not produced in the main mixing line of feed mills and when FDS trucks are used. But, even in cases where these scenarios would be implemented, the risk would not be completely removed given that sources of cross-contamination would still exist at the truck and farm levels and hence should not be overlooked. The model can be used to provide estimations for different levels of AB MF produced and for different policies applied and should then be informed with data after carefully considering the specific situation.

Disclosure of Interest: None Declared

Keywords: cross-contamination, medicated feed, risk assessment

Poster Abstracts

Bacteriology and Bacterial Diseases
ANTIMICROBIAL

PO-PF3-003

Investigation of risk factors for methicillin-resistance in staphylococci on swine farms

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Introduction: Staphylococci are a major disease burden for animals and humans. In swine, *Staphylococcus hyicus* is the causative agent of exudative epidermitis (greasy pig disease) which is characterized by skin lesions and a greasy exudate. Outbreaks of this disease can result in significant morbidity and mortality. Swine are also common carriers of *Staphylococcus aureus* and, although this agent poses little threat to swine, its zoonotic potential is concerning for public health. Both of these staphylococci species have been found to carry the methicillin-resistance gene (*mecA*) which commonly co-locates with the zinc-resistance gene (*czrC*) within a mobile genetic element (SCC*mec*). Given the co-location of these genes, it is predicted that exposure to zinc may co-select for the *mecA* gene in the absence of antibiotics.
Materials and Methods: A cohort study of 390 pigs at 26 farms was completed. Farms were surveyed for demographics, management practices, biosecurity, and antimicrobial usage. Nasal cultures for methicillin-resistant *S. aureus* (MRSA) were completed at weaning and again at 3 weeks post-weaning. Isolates of MRSA were screened for *mecA*, *mecC*, *czrC*, PVL, and phenotypic zinc resistance. Multivariate random-effect logistic regression models were created to analyze the data.
Results: Ninety (23.3%) nursery pigs and 10 (38%) farms tested positive for MRSA. Univariate analysis revealed that MRSA in nursery herds was positively associated with greater stocking densities (P=0.048), disinfection of pens for incoming pigs every time (P=0.022), and use of in-feed zinc ≥2,000 ppm (P=0.022). Multivariate analysis predicted that the odds of MRSA carriage in pigs on a 3,000 ppm zinc diet was 12.4 times greater than the odds of MRSA carriage in pigs on a 250 ppm zinc diet (95% CI: 3.04-50.25; P<0.001). Phenotypic zinc resistance and the *czrC* gene were detected in 36 (90%) and 25 (63%) of the MRSA isolates, respectively. High zinc diets (≥2,000 ppm) were used on 72.7% of the nursery farms. The presence of MRSA was not associated with any particular antibiotic, the number of antibiotics used, or the route of antibiotic administration.
Conclusion: The presence of MRSA in nursery pig herds was epidemiologically associated with the use of high levels of in-feed zinc and this was consistent with the molecular evidence. This evidence supports the theory that zinc can co-select for antibiotic resistance due to the genetic linkage of antibiotic- and zinc-resistance genes within the same genetic element.

Disclosure of Interest: None Declared

Keywords: antibiotic resistance, Staphylococcus aureus, zinc oxide

Bacteriology and Bacterial Diseases
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Characterization of resistance profile of Leptospira interrogans serovar Pomona isolated from swine in Brazil.

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Introduction: The objective of this study is the characterization of antimicrobial sensitivity of 9 *Leptospira interrogans* serovar Pomona isolates. *Leptospira* not only represents a risk to human health, but the study of its susceptibility also lies on the development of its treatment and control in farms to reduce economic losses (e.g. piglets' abortion). Existing studies have used *L. interrogans* human isolates serogroup Icterohaemorrhagiae serovar Icterohaemorrhagiae/Copenhageni to determine strain susceptibility to existing treatments. This study introduces the susceptibility profile of *L. interrogans* serovar Pomona from swine, an important host species.
Materials and Methods: 9 *Leptospira interrogans* Sorovar Pomona were isolated from diseased and apparently healthy swine. For isolation, kidney and abortion samples were collected. For the inoculum, cultures were grown at 30°C for 7 days and then diluted. Broth microdilution was performed and adapted for use with the Sensititre® Standard Susceptibility MIC Plate BOPO6F following Murray and Hospenthal protocol (2004). 50µL of inoculum were added in each Sensititre® MIC Plate well. After 3 days of incubation, 5 µL of 10X alamarBlue® were added to each well. MICs were assessed visually as the lowest antibiotic concentration in the wells without alamarBlue® color change at the 5th incubation day.
Results: Every isolate had high MIC values to tiamulin, gentamicin, chlortetracyclin, oxytetraciclin, neomycin, tilmicosin, trimethoprim/sulfamethoxazole, spectinomycin, sulfadimethoxine. High fluoroquinolone MIC values were also observed. Every isolate appeared to be sensitive to penicillin, ampicillin, ceftiofur, tylosin tartrate and tulathromycin. MIC variability was observed for florfenicol. One isolate presented a slightly different profile with low MIC values for gentamicin, florfenicol, chlortetracyclin and oxytetracycline, while apparently resistant to clindamycin. Clinically, the most important findings are the sensitive strains to β-lactams, the most common treatment for leptospirosis; and MIC values increase for aminoglycosides, tetracyclines and macrolides, widely used in animal production. Our results corroborate previous studies presenting high MIC values for tetracycline, trimethoprim/sulfamethoxazole, sulfadimethoxine, and chloramphenicol/florfenicol. However, higher MIC values for tiamulin, gentamicin, neomycin and spectomycin were unexpected and their veterinary use requires attention.
Conclusion: Pomona strains of this study showed different antimicrobial susceptibility profiles compared to profiles previously described for other strains of L. interrogans. More research is needed to define serovar susceptibility profiles.

Disclosure of Interest: None Declared

Keywords: antimicrobial resistance, Leptospira interrogans, Serovar Pomona

Bacteriology and Bacterial Diseases
ANTIMICROBIAL

PO-PF3-168

Prevalence and antimicrobial resistance profiles of Staphylococcus aureus (SA) recovered from growing pigs in the USA

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Introduction: Although current evidence suggests a relatively low prevalence of MRSA in pigs in the USA, concerns remain about spread of multidrug resistance bacteria from the farm environment to swine workers and the wider community. The aim of this study was to characterize antibiotic resistance profiles of SA isolated from growing pigs in the USA.
Materials and Methods: Twenty nasal swabs were collected from 36 growing pig sites in 11states in the USA, including one known MRSA-positive farm. Antimicrobial susceptibility testing (17 antibiotics) was performed on a subset of 128 SA isolates selected purposively to maximize the diversity by spa type and farm. Susceptibility was based on Clinical Laboratory Standards Institute criteria for all antimicrobials except ceftiofur, tulathromycin and tylosin. To evaluate co-resistance, associations between phenotypic resistance patterns was assessed using Chi square and Fisher's exact test adjusting for multiple comparisons.
Results: MRSA were not detected in any of the pigs except on the positive control farm. Overall SA prevalence was 76% (558/739) and there was considerable diversity found with 33 spa types detected within 4 MLST sequence types: ST9, ST398, ST5 and ST2007. All isolates were resistant to spectinomycin and most were resistant to tetracycline (94.5%), clindamycin (75.0%) and penicillin (both 71.9%). Multiple resistance (>3 antibiotics) was common, and 61 resistance patterns were found among the 128 isolates. The most common resistance profile among isolates was SPE-CTC-OXY-CLI-TIL-FFN.* There were statistically associations found between 10 pairs of antibiotics : TET-CLI, CLI-TIA, CLI-TIL, CLI-FFN, TIL-FFN, TIA-NEO, FFN-NEO,TIL-GEN, GEN-NEO, NEO-ENR. The reported use of antibiotics on farms was poorly correlated with the presence of resistance to the same classes of antibiotics in the isolates studied.
Conclusion: The most striking finding was the apparently low MRSA prevalence in these herds, but a relatively high prevalence of multiple resistant SA phenotypes. Although sample size limited the power of analysis of associations between resistance to antibiotics, many pairs of co-resistance were observed suggesting the potential importance for co-selection of multiple resistance genes as a result of antibiotic use. The public health importance, if any, of multiple resistant SA in the swine reservoir remains to be established.
*Abbreviations: AMP (ampicillin), CTC(chlortetracycline), CLI(clindamycin), ENR(enrofloxacin), FFN(florfenicol), GEN(gentamicin), NEO(neomycin), OXY (oxytetracyline), PEN (penicillin), SPE(spectinomycin), TIA(tiamulin), TIL(tilmocosin)

Disclosure of Interest: None Declared

Keywords: antimicrobial resistance, MRSA, pigs

Bacteriology and Bacterial Diseases
ANTIMICROBIAL

PO-PF3-298

Detection of antibiotic resistant E. coli in weaned piglets

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Introduction: Evaluating the presence of antibiotic resistant (AR) bacteria in swine production is important to estimate the problems associated with bacterial antibiotic resistances on farms. Especially the prevalence of resistance in commensal *E. coli* is a useful indicator of antibiotic resistance in bacteria of different communities. This bacterium causes also diarrhea problems in weaned piglets. For this reason *E. coli* and AR *E. coli* were assessed in weaned piglets of an Austrian farm in the first week after weaning.
Materials and Methods: The prevalence of total *E. coli* and AR *E. coli* was determined in fecal samples of piglets which did not receive antibiotics during the experiment. Experiment started on day 1 with forty weaning pigs, which were split into four groups (= replicates). Individual fecal samples of three pigs per replicate were collected on days 1 and 7 of the experiment. The colony count of *E. coli*, *E. coli* resistant to streptomycin (Str), tetracycline (Tet), sulfamethoxazole (Sul), amoxicillin (Amx), ampicillin (Amp) and extended spectrum ß-lactamases (ESBL)-producing *E. coli* were investigated within 24 hours after collecting the samples. MacConkey (Merck GmbH) agar was used for the detection of total *E. coli,* MacConkey agar supplemented with an antibiotic at the concentration of the CLSI breakpoint for AR *E. coli* and ChromID® ESBL medium for ESBL-producing *E. coli.*
Results: Results showed that the level of *E. coli* in fecal samples was 1.4 x 10^8 cfu/g feces on day 1. Approximately half of these *E. coli* were resistant to Str, whereas Tet-, Sul- or Amx-resistant *E. coli* conformed to one-third of the total *E. coli* count in each case. About 16% or 9.5% of the total *E. coli* count were resistant to Amp or produced ESBL. Thus, it is supposed that multi-resistant *E. coli* were present on the farm. Compared to day 1 a decrease of *E. coli* by 70% was found on day 7 ($p<0.05$). Furthermore the level of Str- and Tet-resistant *E. coli* on day 7 was reduced by 62% and 86%, respectively ($p<0.05$). Although the colony counts of Sul- and Amx-resistant *E. coli* were numerically decreased by 41% and 18% on day 7, these results were not significant. The same applies to the levels of Amp-resistant and ESBL-producing *E. coli,* which numerically increased from day 1 to day 7 by 47% and 94%.
Conclusion: An increase of Amp-resistant as well as ESBL-producing *E. coli* within the first week after weaning was detected in feces of the piglets, while Str- and Tet-resistant *E. coli* numbers decreased. Follow up studies in weaned piglets should be carried out in order to identify reasons for the decrease or increase of resistant *E. coli* in this stressful period.

Disclosure of Interest: None Declared

Keywords: antibiotic resistance, E. coli

Poster Abstracts

Bacteriology and Bacterial Diseases
ANTIMICROBIAL

PO-PF3-184

Local tolerance of two florfenicol injectable solutions in fattening pigs

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Introduction: The local tolerance at injection site is an important parameter for animal welfare and to avoid lesions which may impair carcass quality. The aim of this study was to compare local tolerance of two injectable formulations marketed in Mexico, both containing 400 mg of florfenicol per mL.
Materials and Methods: Fourteen healthy pigs of both sex weighing 82 kg in average were housed in individual pens and randomly allocated to 2 groups receiving one of the 2 formulations tested (group A : Maxflor®L.A, Virbac and group B : Colmax®, Aranda). The pigs had not received any injection in the neck during the preceding month inclusion. Both products were administered according to registered posology (2 intramuscular injections 48 h apart at the florfenicol dose of 15 mg/kg, i.e. 1 ml/26.7 kg). Injections were done 3 inches behind the ear (in left side for the first injection and in right side for the second one) with single use syringes and needles (16Gx1"). All injection sites were scored for swelling, pain, pruritus and size of inflammation zone on 1, 3, 7 and 14 days after administration by clinical examination and thermography. Pigs were slaughtered when withdrawal time of products had elapsed (14 days after second injection). Macroscopic examination of all injection sites was done according to a published grading scale. Samples of all injection sites were taken for histopathology after fixation by formalin, embedding into paraffin then Haematoxylin and Eosin staining of 3-5 µm thin sections. Blinded observations were made for clinical and post mortem phases. The rates of lesions were compared between groups according to Fisher's exact test.
Results: No general or local side effects were recorded on any of the animals. No macroscopic lesions were observed in any of the injection sites. Microscopic lesions were scored on 21% (3/14) of injections sites from group A and 64% (9/14) of injection sites from group B. Lesions in group A consisted in mild to moderate granulomatous infiltration, fibrosis and haemorrhage whereas lesions in group B included also mild to severe necrosis in 57% (8/14) of injection sites, difference between groups being significant (p=0.002).
Conclusion: Our results suggest that both products were well tolerated clinically and did not induce visible macroscopic lesions at injection sites 14 days after the second injection. However, more severe microscopic lesions (particularly necrosis) were observed in group B which may reflect formulations differences.

Disclosure of Interest: None Declared

Keywords: Florfenicol, Injection, Tolerance

Bacteriology and Bacterial Diseases
ANTIMICROBIAL

PO-PF3-080

Minimum Inhibitory and Minimum Bactericidal Concentrations of Gamithromycin against European swine respiratory bacterial field isolates

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Introduction: Respiratory infections remain a significant problem for the swine industry and result in substantial economic loss. Swine Respiratory Disease (SRD) is a complex condition involving viral agents and bacterial agents such as *Actinobacillus pleuropneumoniae (App)*, *Haemophilus parasuis (Hp), Bordetella bronchiseptica (Bb)* and *Pasteurella multocida (Pm).*
The *in vitro* activity of gamithromycin, macrolide sub-class, 7a-azalide antimicrobial, was evaluated against common field strains isolated from outbreaks of clinical disease across Europe.
Materials and Methods: A total of 358 isolates (100 *Pm*, 100 *App*, 91 *Bb* and 67 *Hp*) were sourced from the VETPATH III European collection coordinated by the European Animal Health Study Centre (Centre Européen d'Etudes pour la Santé Animale; CEESA). The strains were isolated between 2010 and 2012 in eight European Countries (Belgium, Denmark, France, Germany, The Netherlands, Poland, Spain and the UK) and exclusively originated from clinically sick pigs aged from 3 weeks to 6 months and showing depression, hyperthermia (>39.8°C), with one or more of these respiratory signs: polypnea, dyspnea, cough and/or sneezing. The animals had not been exposed to any form of antimicrobial treatment for at least 15 days prior to sample collection. The isolates were epidemiologically unrelated (one isolate per outbreak, per farm without resampling within a six month period).
Minimum Inhibitory concentration (MIC) and Minimum Bactericidal Concentrations (MBC) were generated using harmonized and internationally recognized broth microdilution methods published by the Clinical & Laboratory Standards Institute (CLSI) in guidelines M100-S23 (2013), VET01-A4 (2013) and M26A (1999) . MIC values were determined for the whole collection and MBC values were assessed for half of the isolates (50 *Pm*, 50 *App*, 46 *Bb*, 67 *Hp*) selected at random within each MIC category.
Results: The MIC ranges were 0.25-2 µg/mL for Pm, 2-16 µg/mL for App, 1-4 µg/mL for Bb and 0.06-4 µg/mL for Hp. MIC50 and MIC90 of the isolates occurred at 0.5/1 µg/mL, 4/4 µg/mL, 1/2 µg/mL and 0.25/0.5 µg/mL respectively. MBC killing of 50% and 90% of the isolates occurred at 1/2 µg/mL for Pm, 2/4 µg/mL for App, 2/4 µg/mL for Bb and 0.5/0.5 µg/mL for Hp. For the majority of the isolates (167/181), MBC ≤ 2 x MIC. Only 14 isolates out of 181 showed MBC > 2 x MIC
Conclusion: MIC and MBC for gamithromycin were determined for recent swine bacterial respiratory pathogens representative of distinct areas of the EU. This study revealed a very high susceptibility of all the tested strains (*App*, *Hp, Bb* and *Pm)* to gamithromycin and evidenced a clear bacteriostatic and predominantly bactericidal effect on these SRD pathogens.

Disclosure of Interest: A. Richard-Mazet Conflict with: MERIAL S.A.S., J. Hayward: None Declared, E. Siegwart: None Declared, A. Pfefferkorn Conflict with: MERIAL S.A.S., P. Dumont Conflict with: MERIAL S.A.S.

Keywords: Gamithromycin, Minimum Bactericidal Concentration, Minimum Inhibitory Concentration

Bacteriology and Bacterial Diseases
ANTIMICROBIAL

PO-PF3-131

Pharmacokinetics of gamithromycin in swine plasma and bronchoalveolar cells

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Introduction: Gamithromycin, the active pharmaceutical ingredient in Zactran® 15% w/v gamithromycin injection from Merial, is a novel azalide antimicrobial developed for the treatment of swine respiratory disease. Gamithromycin is a 15-membered semi-synthetic macrolide with a uniquely positioned alkylated nitrogen at the 7a-position of the lactone ring.
The PK properties and dose proportionality of gamithromycin in swine plasma were determined after a single intravenous (IV) dose of 6 mg/kg or intramuscular (IM) injection at 3, 6, 12 mg/kg body weight (BW). Additionally, gamithromycin concentration in bronchoalveolar (BAL) cells was determined in another study following single IM administration of gamithromycin at 6 mg/kg BW for better understanding of the PK/PD relationships.
Materials and Methods: A total of 28 healthy pigs were randomly assigned to 5 groups to study plasma PK and dose proportionality. Group 1 had 2 pigs as Control, Group 2 had 8 pigs dosed IV at 6 mg/kg BW, and Groups 3-5 consisted 6 pigs dosed once IM at 3, 6, 12 mg/kg BW, respectively.
To determine gamithromycin concentrations in BAL cells, 36 healthy pigs were allocated to 11 groups. Six pigs served as untreated controls. Pigs in 10 groups (n=3 each) were administered a single IM dose of 6 mg/kg BW on Day 0. BAL cells were sampled at various time points after dosing.
The collected plasma and BAL samples were analyzed using validated methods with tandem mass spectrometry.
Results: Gamithromycin was rapidly absorbed into the systemic circulation and distributed to tissues quickly. Maximum gamithromycin plasma concentration was 960 ng/mL at 0.083-0.25 hours (h) with a terminal half-life of 94.1 h in pigs dosed IM at 6 mg/kg BW. Volume distribution and clearance were 39.2 L/kg and 1030 mL/kg/h, respectively. The absolute bioavailability was 92.2%. Dose proportionality was observed with AUCs.
Maximum gamithromycin concentration of 20.5 µg/mL was achieved in BAL cells at 24 h after dose. Terminal half-life was 87.1 h in BAL cells. The maximum ratio of gamithromycin concentration in BAL cells over in plasma was 1870 at 5 days post dose.
Conclusion: Gamithromycin was well absorbed after IM administration to pigs. Dose proportionality was established over the range of 0.5x to 2.0x the recommended dosage of 6 mg/kg BW. Based on minimum inhibitory concentrations of gamithromycin for target pathogens, potential therapeutic concentrations was achieved in 30 min after dose and persisted for > 6-10 days.
Zactran® is a registered trademark of Merial, Inc.

Disclosure of Interest: R. Huang Conflict with: MERIAL Inc., D. Hamel Conflict with: MERIAL GmbH, R. Tessman Conflict with: MERIAL Inc., A. Mullins Conflict with: MERIAL Inc., T. Malinski Conflict with: MERIAL Inc., S. Rehbein Conflict with: MERIAL GmbH, L. Letendre Conflict with: MERIAL Inc.

Keywords: Gamithromycin, Pharmacokinetics, Swine Respiratory Disease

Bacteriology and Bacterial Diseases
ANTIMICROBIAL

PO-PF3-302

Supplementation with Clostridium butyricum (Miya-Gold® S) reduces antimicrobial consumption

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Introduction: The Danish Ministry of Agriculture monitors antimicrobial use by setting limits for the amount of average daily doses (ADD) used in farm animals. For pig farms producing 7-30 kg pigs the limit is 22,9 ADDs since November 2014. The evolution in antimicrobial use measured in ADDs is described in a 7-30 kg weaner pig farm before and after supplementation of a probiotic (Miya-Gold® S).
Materials and Methods: The Danish Ministry of Agriculture monitors antimicrobial consumption at herd level in a central database, Vetstat. All antimicrobials are under prescription and reported. The Ministry defines the ADD as mg active product/kg body weight treated. In 7-30 kg pig production the ADDs are standardized to 15 kg bodyweight. The case farm producing 35.000 7-30 kg pigs from own sow herd and vaccinates for *Mycoplasma hyopneumoniae* and *Porcine Circovirus Virus* type 2. Pigs were severely affected by enteritis causing high mortality by *Enterobactericea* until Miya-Gold® S was supplemented from week 28 (first week of July) at 2 kg/MT in the first feed (pigs at 6,2 kg), 1 kg/MT in the second feed, 0,5 kg/MT in the third feed. Antimicrobial consumption reported as ADDs prescribed each month from October 2014 to June 2015 (9 months before supplementation) was compared to 6 months after the start of supplementing Miya-Gold® S (July 2015 to December 2015) by students t-test. Also weekly mortalities were recorded and compared from week 24-27 (4 weeks before supplementation) to week 28-36 (9 weeks during supplementation) by students t-test. Pigs are held 56 days at the site on average.
Results: Monthly antimicrobial prescription has dropped from 16,55 ADDs 9 months before to 7,94 ADDs 6 months after the start of Miya-Gold® S supplementation (p≤0,00016). Prescription of antimicrobials for gut health decreased from 8,43 ADDs to 1,98 ADD (p≤0,03). Weekly mortalities dropped from 1,5% 4 weeks before to 0,3% during the 22 weeks after the start of Miya-Gold® supplementation (p=<0,000000003). Thus, total average mortality 4 weeks before supplementation was 12% for 7-30 kg pigs, after supplementation of the probiotic the mortality dropped to 2.4%.
Conclusion: Control of gut health is essential for lowering antimicrobial consumption. Miya-Gold® S is a zootechnical feed additive based on spores of *Clostridium butyricum* that germinate in the ileum and colon of the pig and produce butyric and acetic acid stabilizing the microflora of the pig. This case describes a lowering of antimicrobial use by 50% by controlling gut health with Miya-Gold® S, and restoring weekly mortalities to normal levels in pigs from 7-30 kg.

Disclosure of Interest: None Declared

Keywords: antimicrobial use, Clostridium butyricum, pig

Poster Abstracts

Bacteriology and Bacterial Diseases
ANTIMICROBIAL

PO-PF3-240

Pharmacokinetic profile of Apravet® after oral administration

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Introduction: The objective of this study was to measure the concentrations of apramycin in the plasma and in the gastrointestinal tract during treatment with Apravet® and to determine the maintance of apramycin (APRM) after stopping the treatment.

Materials and Methods: Five groups of 4 pigs (Danube white), of both sexes (8.8-10.5 kg), 4-5 weeks of age, were used. On day 0 of the trial, four groups were treated via feed with 8 mg per kg bodyweight (BW) APRM as Apravet® 100 g/kg premix for 21 consecutive days. One group was not treated (Control group). Samples of the plasma, small intestinal content and large intestinal content were taken at day 21 (during treatment) and 12, 24, 36 and 48 hours after stopping the treatment. The evolution of concentrations of APRM was assessed applying HPLC determination in plasma and intestinal contents of pigs. the results of the examination were determined according to t-test of Student-Fisher.
Results: The concentrations of APRM in the plasma (PC) and the small (SIC) and large intestinal (LIC) contents on day 21 were respectively 0.162 µg/ml and 45.0 and 88.2 µg/g. Twelve and 24 hours after the end of the treatment, the concentration remained high in the intestinal tract, respectively 33.2 and 10 µg/g (SIC) and 54 and 23 µg/g (LIC). The plasma concentrations quickly declined after 24 hours under LOQ (<0.05). No statistically significant difference in APRM concentrations in small and large intestines were found, 12 hours after the treatment as compared to those found on the 21st day of the treatment. At all other time points APRM concentrations were statistically lower versus those found on the 21st day of the treatment
Conclusion: The results from the study show that Apravet® administration via feed at 8 mg per kg BW concentrates in small and large intestines which may have a significant role in establishing PK/PD relationships and clinical efficacy against bacterial enteritis in pigs. The present study highlights the maintenance of APRM concentrations in in the small and large intestines up to 36 hours after the 21st day of treatment. Until now, clinical breakpoints for apramycin used to treat bacterial enteritis were based upon aminoglycosides administered via parenteral route from other species. This study might help to better predict efficacy *in vivo* based upon *in vitro* susceptibility testing

Disclosure of Interest: None Declared

Keywords: apramycin pharmacokinetics oral

Bacteriology and Bacterial Diseases
ANTIMICROBIAL

PO-PF3-139

Second-line therapeutic efficacy of ZACTRAN® (gamithromycin injectable solution) against Swine Respiratory Disease in a field trial in Japan

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Introduction: Swine Respiratory Disease (SRD) is a complex condition involving viral agents and bacterial agents such as *A. pleuropneumoniae* , *H. parasuis* and *P. multocida*. These organisms often act together to increase the severity and duration of the disease, therefore, appropriate control of these pathogens is important for swine health management. The present abstract refers to the field efficacy of ZACTRAN®, Merial in comparison with ADVOCIN® (Danofloxacin mesylate injectable solution), Zeotis when used as a second-line therapy for the treatment of SRD in pigs that had failed to respond to first line therapy.
Materials and Methods: The study was conducted on 2 Japanese farms. Each site was recruited based on the confirmation of SRD clinical signs associated with lesions and isolation of the target pathogens in lung tissue at necropsy from 5 sentinel pigs per site.
A total of 63 3 month-old pigs weighing 21.5 to 43 kg were recruited on Day 0 (D0). The included pigs were from the same batch on each test site. These pigs displayed a minimum level of a composite SRD clinical score for respiratory condition, cough, physical activity and appetite and a body temperature of at least 39.5°C on D-7, D-5/D-4 and on D0 just prior to treatment allocation. They has failed to respond to treatment with either an amoxicillin therapy (15 mg/kg bodyweight (BW), on D-7 and D-5) or oxytetracycline (20 mg/kg BW, on D-7 and D-4).
Allocation to the treatment groups was performed randomly in replicates of 3 pigs in a 2:1 ratio of ZACTRAN-treated pigs to ADVOCIN-treated pigs. On D0, ZACTRAN was administered IM at 6.0 mg/kg BW once; ADVOCIN was injected once daily for 3 consecutive days IM at 1.25 mg/kg BW.
Pigs were monitored daily for their clinical scores until D14. A clinical improvement index was calculated for each pig using the clinical scores recorded on D0 and D7. The proportion of improved pigs for each treatment was compared using a non-inferiority hypothesis test (non-inf. margin = 0.15).
Results: No health abnormality other than SRD related symptoms was observed in any pigs treated with ZACTRAN. No local reactions were observed in any ZACTRAN-treated pigs. The proportion of improved pigs was 86% in ZACTRAN-single-treated pigs and 65% in ADVOCIN-three-time-treated pigs. This result supported that on single injection of ZACTRAN was equivalent to or better than three consecutive injections of ADVOCIN, a reference product approved for the treatment of SRD.
Conclusion: Under the conditions of this trial, a single IM injection of ZACTRAN was shown to be effective for the second-line treatment of pigs with spontaneously acquired SRD that had failed to respond to first line treatment by providing clinical cure of 86%.

Disclosure of Interest: Y. Kondo Conflict with: Merial Japan Limited, N. Nakanishi: None Declared, Y. Wakui Conflict with: Merial Japan Limited, A. Richard-Mazet Conflict with: Merial S.A.S., K. Tokuyama Conflict with: Merial Japan Limited, G. Kinoshita Conflict with: Merial Japan Limited, P. Jeannin Conflict with: Merial S.A.S.

Keywords: Gamithromycin, macrolide, Swine Respiratory Disease

Bacteriology and Bacterial Diseases
ANTIMICROBIAL

PO-PF3-037

Antibiotic Benchmarking In Canada: Comparison of Data over Time at the Prairie Swine Center

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Introduction: Benchmarking data using computer software programs has been integrated into the swine industry for decades to provide an opportunity for improved management. Antibiotic benchmarking is a relatively new production tool being introduced to swine herds in Canada. Antibiotic benchmarking provides an opportunity for objective discussion between the veterinarian and producers in prudent use of antibiotics including selection of product, usage, dosage, timing and opportunity for improvement. There are a variety of methodologies for analysing farm data but consistency in both method and interpretation it is important to accurately report outcomes and provide comparisons.
Materials and Methods: Benchmarking took place at the Prairie Swine Centre (PSC), a 340 sow (PIC) farrow-to-finish research farm, near Saskatoon, Saskatchewan, Canada. The breeding herd is closed with replacement gilts produced through internal multiplication. A comprehensive internal and external biosecurity program ensures a high health status is maintained. Productivity exceeds 80% of other commercial swine units.
Antibiotic use and farm animal inventory for a full year (September/2014 to September/2015) were collected from the farm for analysis. These results were compared to 2013 usage. The Compass calculator developed by Boehringer Ingelheim was used to calculate standardized antibiotic usage. This is reported as animal daily dose/100 animal days (ADD/100 animal days) and as "weight of active molecule-mg./ kg of pork produced". Four production stages were identified; sows, suckling piglets, nursery pigs (wean to 30 kg), and grow-finishers (markets). The following information was collected; antibiotic used, dosage, stage of production, treatment route, drug class, preventative vs curative, average exit weight (kg), days at risk, and number of animals treated.
Results: Total antibiotic use in 2015 was 1.52 mg/kg versus 33.5 mg/kg in 2013. After 2013 two major changes were made; all dietary feed medications were removed and sow lameness protocols were updated. For 2015, 100% of medications used are now curative and injectable versus only 52% of medications being injectable and 48% being in feed in 2013; ADD/100 animal days in 2015 vs. 2013 for nursery – 0.15 vs 37.16 and finisher- 0.05 vs 1.86.
Conclusion: As an early adopter of antibiotic benchmarking technology the PSC has taken a leadership role in the prudent use of antibiotics; effectively reducing their use without compromising welfare. The "on farm" approach to benchmarking is a useful tool in assessing, evaluating and appropriately reducing antibiotic use in swine units.

Disclosure of Interest: None Declared

Keywords: Antimicrobial benchmarking, Antimicrobial reduction, antimicrobial usage

Bacteriology and Bacterial Diseases
ANTIMICROBIAL

PO-PF3-283

Antibacterial Activities of Lactic Acid Bacteria Probiotics Derived from Pig Feces in Thailand

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Introduction: Probiotic has been used as an alternative for antibiotic replacement that possesses an antibacterial efficacy using in pig industry. Lactic acid bacteria (LAB) are the important probiotic members used worldwide. However, a limitation of probiotic use is mainly caused by host specificity and appropriateness of geographical difference. Besides, the target of probiotic against enteric pathogens should be considered upon epidemiological evidence. However, there was still a lack of antibacterial activity between the local GI pathogen in Thailand and probiotic candidates ensuring their efficacies, *in vitro*. The purpose of study was to evaluate antibacterial activities of seven strains of LAB isolated from pig feces in Thailand against Enterotoxigenic *Escherichia coli* (ETEC), Enterohaemorrhagic *Escherichia coli* (EHEC) and *Salmonella* Cholerasuis isolated from clinical piglets.
Materials and Methods: The indicator pathogenic bacteria consisting of 5 EHEC, 5 ETEC, 5 *S.* Cholerasuis, *E. coli* ATCC 25922 and *S.* Thyphimurium ATCC 13311 were added to a 20 ml of 42° C Mueller-Hinton agar to a final concentration of 10^5 CFU/ml and poured into Petri dish, then 6 mm-diameter wells were punched into the surface using a sterile borer. Seven LAB strains; 3 *Lactobacillus plantarum*, 2 *Enterococcus faecium*, 1 *Pediococcus acidilactici* and 1 *Pediococcus pentosaceus* have been screened using high performance of probiotic characteristics. Cell-free culture supernatants (CFCS) of the selected LAB grown in MRS broth at 37 °C for 48 h were obtained by centrifugation (5,000 rpm, 4 °C, 15 min) and filtered through a 0.22 µm filter. A 50 µl CFCS was measured pH, added to each well of the plates and incubated at 37 °C for 18 h. The antimicrobial activity was determined by diameter of the inhibition zone around the wells.
Results: All LAB were further characterized by tolerance to low pH and bile environment, and adhesion to cell line from the previous study. CFCS of tested lactobacilli and pediococci strongly presented antimicrobial activity against all pathogenic bacteria by presenting the clear zone. In contrast, no strain of *E. faecium* presented antimicrobial activity. The selected lactobacilli showed the best antimicrobial activity comparing with the others ($P \leq 0.05$). Moreover, we observed antimicrobial activity from supernatants those possessed pH below 4.
Conclusion: We confirmed the antibacterial efficacy of lactobacilli and pediococci field strains against common cause of diarrhea in piglets, *in vitro*. According to their properties, the native LAB strains as a good probiotic candidate will be further studied for time kill assay and clinical efficacy, *in vivo*.

Disclosure of Interest: None Declared

Keywords: Antibacterial activity, Pig feces, Probiotics

Poster Abstracts

Bacteriology and Bacterial Diseases
ANTIMICROBIAL

PO-PF3-061

Determination of minimum inhibitory concentrations of gamithromycin against European swine food-borne pathogen and commensal intestinal field isolates

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Introduction: To address concerns of antibiotic (AB) resistance in both human and animal health, approval of veterinary antibiotics requires an assessment for potential impact on resistance development in food-borne pathogens (FBPs). During treatment for Swine Respiratory Disease (SRD), populations of FBPs and commensal organisms from the genera *Escherichia coli (Ec)*, *Salmonella (Sa)*, *Enterococcus (En)* and *Campylobacter (Cb)* may be impacted. Isolates from the respective genera may vary in susceptibility to various ABs, depending on selective pressures in place at the time of collection. Susceptibility is measured by determining the Minimum Inhibitory Concentration values (MICs) for each isolate against the test antibiotic. The risk for development of co- or cross-resistance is assessed by comparing MICs for gamithromycin (GAM) with other ABs. The *in vitro* activity of GAM, a macrolide of the sub-class azalide, was evaluated against European swine intestinal isolates taken at the time of slaughter.
Materials and Methods: 240 isolates (60 isolates each of Ec, Sa, En and Cb) were sourced from the EASSA III collection coordinated by the European Animal Health Study Centre (CEESA). Recent isolates were collected from healthy swine in eight European Countries (Belgium, Denmark, France, Germany, Hungary, Netherlands, Spain and UK). MICs were generated using Clinical & Laboratory Standards Institute (CLSI) methods (VET01-A4, 2013). Based on these results, 52 isolates (12 *Ec*, 16 *Sa*, 14 *En*, 10 *Cb*) were evaluated for co- and cross-resistance using GAM and a panel of 13 common ABs.
Results: The GAM MIC distribution (in µg/mL) for *Ec* was 2-8; for 95% of *Sa* isolates (4-8); *En* (0.12 to >32); and 94% of *Cb* (either 0.06-0.25 or >32). Resistance patterns of isolates were evaluated using isolates with high MICs. GAM MICs were strongly correlated to other macrolides (erythromycin, azithromycin, tulathromycin) and lincomycin. All *En* and *Sa* isolates with raised GAM MICs were resistant to tetracycline. No correlation was seen between the MIC of GAM and any of the other non-macrolide ABs. The few isolates with raised GAM MICs did not demonstrate linked co-resistance with other antimicrobial classes in the test panel.
Conclusion: MICs for GAM were determined for recent swine food borne pathogens and commensals isolated from the intestine of healthy swine from distinct areas of the EU. This study revealed a very high susceptibility of *Ec* and *Sa* to GAM, along with expected susceptibility patterns to *En* and *Cb*. Based on these studies and literature, the use of GAM to treat SRD is not considered to influence development of resistance and will have no impact on human food safety.

Disclosure of Interest: J. Harriman Conflict with: Merial, Inc., A. Richard-Mazet Conflict with: Merial S.A.S., J. Hayward: None Declared, P. Silley: None Declared, J. Gerhart Conflict with: Merial, Inc.

Keywords: antibiotic resistance, macrolide, Swine Respiratory Disease

Bacteriology and Bacterial Diseases
ANTIMICROBIAL

PO-PF3-219

Effect of cefquinome treatment onto tonsillar colonization with Streptococcus suis

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Introduction: Healthy pigs carrying *Streptococcus (S.) suis* on their tonsils are known to be a source of *S. suis* transmission in pig herds. *S. suis* can cause severe clinical disease, especially if co-infection with other pathogens occur. As a precautionary measure in experimental infection trials with other pathogens, pigs are treated with antibiotics to eliminate as much as potentially pathogenic bacteria as possible. The effect of cefquinome treatment was evaluated for pigs colonized with *S. suis*.
Materials and Methods: Thirty clinically healthy ten-week-old crossbred piglets from a herd unsuspicious for PRRSV and SIV, but with a prehistory of *S. suis* serotype 2 -associated diseases such as meningitis, arthritis and sudden deaths, were selected for an infection trial with influenza A virus. After the pigs' arrival at the isolation facilities tonsillar swabs were immediately taken from all animals and submitted for bacteriological examination. Following, antibiotic susceptibility of recovered *S. suis* isolates were analyzed by agar disk diffusion test. Accordingly to test results, all pigs were treated intramuscularly on three consecutive days with cefquinome (Cobactan® 2,5 %, 2mg/kg BW). Five days after the last treatment tonsillar swabs were again tested for *S. suis*. All isolates were characterized by multiplex PCR targeting *S. suis* housekeeping gene *gdh,* capsular genes for the differentiation of serotypes 1, 2, 7 and 9, as well as genes for virulence-associated factors *epf*, *mrp*, *sly* and *arc*A.
Results: Pigs stayed healthy during the examination period. *S. suis* was isolated from tonsillar swabs prior to treatment from 25 out of 30 piglets (83 %), but isolates did not belong to serotypes 1, 2, 7 or 9. All isolates were negative for *epf*, *mrp* and *sly*, but were positive for *arc*A. After treatment still 16 out of 30 tonsillar swabs were tested positive for *S. suis* (53 %). Two of them were tested negative at first examination but positive after treatment. However, a semi-quantitative assessment of the bacterial burden resulted in a significant reduction of bacterial growth after treatment.
Conclusion: It was not possible to eliminate *S. suis* consistently from tonsillar tissue, but bacterial load was significantly reduced. *S. suis* strains isolated in this study were susceptible to cefquinome *in vitro,* but adequate antibacterial concentrations might not be achieved in tonsillar tissue.

Disclosure of Interest: None Declared

Keywords: Cephalosporins, streptococcus suis, tonsillar colonization

Bacteriology and Bacterial Diseases
ANTIMICROBIAL

PO-PF3-054

Consumption of antimicrobials in Finnish swine herds 2014

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Introduction: This abstract describes usage of antimicrobials in Finnish swine herds in 2014.
Materials and Methods: In 2014 Finnish swine health program (Sikava) collected information from its system from 1282 pig herds. This data consists of 250 519 recordings from 1204 farms.
Results: The most commonly administered antimicrobial was procaine benzylpenicillin. It´s active ingredient, benzylpenicillin, was used 1199 kg. The most common diagnoses for using penicillin were arthritis and other leg problems in sows and piglets. In finishers the most common diagnosis was tail biting. Chlortetracycline reached 1138 kg. It was used only in respiratory and intestinal infections, of which the former was the most common indication. Sulfonamides combined with trimethoprim were used altogether 290 kg. The most common diagnosis was enteritis in weaners and metritis, PDS (MMA) or urinary infection in sows. The rest of the used antimicrobials in the order of usage were amoxicillin (143 kg), oxytetracyclin (88 kg), tylvalosin (80 kg), tiamulin (26 kg), tylosin (20 kg), ampicillin (20 kg), lincomycin (15 kg), enrofloxacin (2 kg), marbofloxacin (0,4 kg) and danofloxacin (0,4 kg). Kefalosporines were not used at all.
Conclusion: Finnish Food Safety Authority Evira has given national recommendations for the use of antimicrobials against the most common infectious diseases in animals in 2009. For arthritis and other leg problems Evira recommends to use penicillin G as the first choice. This recommendation is well adapted in Finnish pig farms. Evira recommends to treat tail biting with penicillin G and this is also the common practice. For respiratory and intestinal disorders Evira recommends to select the antimicrobial according to the bacteriological finding. In respiratory disorders penicillin G targeting actinobacillosis and pasteurella infections is the recommendation as the first choice. The use of penicillin in respiratory infections is not adapted into practice most likely because of its impractical administration parenterally to large groups. For intestinal disorders chlortetracycline is not the first drug choice and still it was widely used for enteritis. Metritis, urinary infections and PDS (MMA) are recommended to be treated with sulfonamides or aminopenicillins and they are implemented very well.
This data covers most of the Finnish pig production and is therefore well applicable in most of the herds. In Finland, the usage of antimicrobials is concentrated on using narrow spectrum antimicrobials in most cases. The national recommendations for most of the animal diseases are well followed. Respiratory and intestinal infections are commonly treated with tetracyclines, which is not according to recommendations.

Disclosure of Interest: None Declared

Keywords: antimicrobial usage, benzylpenicillin, chlortetracycline

Bacteriology and Bacterial Diseases
ANTIMICROBIAL

PO-PF3-010

BAYTRIL® RESISTANCE MONITORING 2010-2015:
ANTIMICROBIAL SUSCEPTIBILITY OF PORCINE PATHOGENS IN GERMANY

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Introduction: Antimicrobial resistance is a concern in the antimicrobial therapy of both humans and animals. Knowledge on the actual susceptibility and its development over the years is important for ensuring long-term antimicrobial efficacy. Therefore, in the early nineties of the past century Bayer has established a susceptibility monitoring program for target animal pathogens obtained from food producing animals in Germany. Here, the susceptibility status for enrofloxacin, the active ingredient of Baytril®, is presented with regard to respiratory pathogens recovered from pigs.
Materials and Methods: Bacteria were isolated by three diagnostic laboratories from samples of diseased pigs during 2010-2015 across Germany. Per farm and outbreak only one isolate was included. The Minimum Inhibitory Concentrations (MICs) of enrofloxacin were determined by agar dilution methodology according to the Clinical and Laboratory Standards Institute (CLSI) in a central lab. Enrofloxacin resistance was calculated using the CLSI breakpoints of ≥ 1 µg/mL for *Pasteurella multocida* (PM) and *Actinobacillus pleuropneumoniae* (APP), and ≥ 2 µg/mL for *Streptococcus suis* (SS). For pathogens for which CLSI breakpoints have not been set (*Bordetella bronchiseptica,* BB *and Haemophilus parasuis,* HP), a tentative breakpoint of ≥ 2 µg/mL was applied.
Results: In total, 606 isolates were tested. The most common species isolated from respiratory tract samples were PM (n=181), BB (n=120), SS (n=89), APP (n=79) and HP (n=41). In addition, other species (n=96) were found in lower numbers, e.g., *Trueperella pyogenes, Staphylococcus* spp., *Klebsiella* spp., *Pseudomonas aeruginosa, Streptococcus* spp. and *Escherichia coli.* Due to the low numbers per species, these isolates were not further analyzed. $MIC_{50/90}$ values were 0.008/0.03 for PM, 0.5/1 for BB and SS, 0.06/0.125 for APP and 0.015/0.03 for HP. For PM, APP and HP no resistant isolates were found. For BB and SS the mean resistance rates were 1.7 % and 2.2%, respectively.
Conclusion: This survey demonstrates a high susceptibility of major respiratory pathogens obtained from German pigs to enrofloxacin after more than two decades of therapeutic use of fluoroquinolones in veterinary medicine. Results are consistent with findings of other national and European monitoring surveys, e.g. GE*RM*-Vet (Germany) and VetPath (Europe). In spite of this high susceptibility, prudent and responsible use of fluoroquinolones as well as resistance monitoring are imperative.

Disclosure of Interest: C. Ludwig Conflict with: Employee of Bayer Animal Health GmbH, S. Fälker Conflict with: Employee of Bayer Animal Health GmbH, C. Bhushan Conflict with: Employee of Bayer Animal Health GmbH, A. de Jong Conflict with: Employee of Bayer Animal Health GmbH, S. Tennagels Conflict with: Employee of Bayer Animal Health GmbH, B. Stephan Conflict with: Employee of Bayer Animal Health GmbH

Keywords: Enrofloxacin, Germany, Susceptibility

Poster Abstracts

Bacteriology and Bacterial Diseases
ANTIMICROBIAL

PO-PF3-029

In vitro susceptibility of porcine isolates of Clostrdium perfringens to selected antimicrobials

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Introduction: Increasing antimicrobial resistance is one of the major problems concerning both veterinary medicine and human health. There are but few reports on antimicrobial susceptibility of *C. perfringens* from pigs. The aim of this study was to evaluate the susceptibility of porcine isolates of *C. perfringens* to antimicrobial agents commonly used in swine diseases therapy.
Materials and Methods: A total of 159 *C. perfringens* isolates were obtained from both diarrheic and healthy: piglets, weaned pigs and fatteners. The isolates were collected in the National Veterinary Research Institute (NVRI) over the period of 2008-2015.
The minimum inhibitory concentration (MIC) of amoxicillin/clavulanic acid, ampicillin, ceftiofur, colistin, enrofloxacin, florfenicol, gentamicin, neomycin, spectinomycin, oxytetracycline, tylosin, tiamulin and trimethoprim/sulphamethoxazole was determined by broth microdilution using Sensititre plates (TREK Diagnostics).
The test and the classification of the strains were carried out in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines and European Committee on Antimicrobial Susceptibility Testing (EUCAST). For each antimicrobial, the range of results, MIC50 and MIC90 were calculated.
Results: More than 80% of isolates had MIC value <0.5 µg/mL of amoxicillin/clavulanic acid and ampicillin. Almost 90% of isolates had MIC value <1 µg/mL of florfenicol. Over 90% of isolates had MIC value >64 of neomycin and almost 75% had MIC value >16 of gentamicin. MIC50 and MIC90 of trimethoprim/sulphamethoxazole were 32 and 64, respectively.
Important differences between MIC50 and MIC90 were noticed (≥ 5-fold dilutions) regarding ampicillin (MIC50/MIC90 – <0.5/128 µg/mL), enrofloxacin (MIC50/MIC90 – 0.25/16 µg/mL), tylosin (MIC50/MIC90 – <0.25/>128 µg/mL) and tiamulin (MIC50/MIC90 – 2/>128 µg/mL), revealing variability of the isolates as far as antimicrobial susceptibility is concerned.
Conclusion: The results of our study confirm a high sensitivity of *C. perfringens* isolated from pigs to beta-lactams. However, increasing resistance to this group of antimicrobials has also been observed in this study.
Both the MIC50 and MIC90 values for florfenicol were low. These results suggest that *in vivo* resistance exists in a slight proportion of the isolates.
Studies with other domestic species indicate a high degree of susceptibility of *C. perfringens* isolates to this antibiotic. Neomycin, gentamicin and trimethoprim/sulphamethoxazole had high MICs, suggesting poor efficacy of these antibiotics against *C. perfringens* isolates from pigs; this phenomenon was reported previously by other researchers.

Disclosure of Interest: None Declared

Keywords: Antimicrobial susceptibility, Clostrdium perfringens, Minimum Inhibitory Concentration

Bacteriology and Bacterial Diseases
ANTIMICROBIAL

PO-PF3-299

Field studies of a new probiotic composition (AQ1202-PigLife) for newborn piglets

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Introduction: The objective of this study was to evaluate the effect of providing a special feed preparation with a new feed additive based on probiotics to newborn piglets in the first day of life, on their performance during the lactation period. Oral administration of porcine lactic acid lactic bacteria in the first hours of life permits an early colonization of the intestine by beneficial microorganisms that can hinder or prevent a later colonization by pathogens. An appropriate and early intestinal microbiota influences and modulates the maturation of both innate and acquired immunity.
Materials and Methods: PigLife is an oral suspension that is composed of two freeze-dried lactic acid bacteria selected by their probiotic capabilities from a collection of isolates of colostrum of sows and meconium of piglets. Both strains are QPS (Qualified Presumption of Safety) according to EFSA requirements. This product is intended to be administered in one 2 ml dose as soon as possible after birth.
The efficacy of PigLife was tested in four different conventional Spanish pig farms, eventually in several experiments, during 2014 and 2015. In every farm, new born piglets were divided in a group treated with the probiotic (AQ1202-PigLife) and a control group.
Litters in both groups were monitored from farrowing to weaning. Productive and clinical data were recorded and the quantification of the main bacterial groups of the intestinal microbiota in the faeces of piglets was performed by qPCR when possible.
Results: Mortality and antibiotic treatments were significantly lower in piglets treated with PigLife. In addition, both mild and severe diarrhea were recorded more frequently in control litters and in some cases significant differences were found in the weaning weight of piglets.
The quantification of microbiota revealed a better balance of beneficial bacterial groups in the faecal samples of probiotic treated piglets.
Conclusion: The treatment with PigLife reduces mortality, diarrhoea and the need of antibiotic treatments in lactating piglets. Besides, it should be expected that a better balanced microbiota contribute to improve health and production results in weaning and fattening periods.

Disclosure of Interest: None Declared

Keywords: Microbiota, piglets, Probiotic

Bacteriology and Bacterial Diseases
BRACHYSPIRA

PO-PF3-178

Antimicrobial susceptibility of Finnish Brachyspira pilosicoli isolates

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Introduction: *Brachyspira pilosicoli* is the etiologic agent of porcine colonic spirochetosis, a diarrheal disease in growing pigs. Colitis associated with *Brachyspira pilosicoli* is less severe than swine dysentery caused by *Brachyspira hyodysenteriae*, however, antimicrobial therapy is needed on some farms to treat diarrhea in weaners and in young finishing pigs. According to principles of prudent use of antimicrobials, only effective drugs should be used. Resistance to lincomycin and especially to tylosin was very widespread in Finnish *Brachyspira pilosicoli* isolates already during 1996-1998. Decreased susceptibility to tiamulin was reported in some porcine *B. pilosicoli* isolates in Finland in the 1990's and decreased susceptibility to tiamulin has been reported in Swedish *B. pilosicoli* isolates. This study reports the in vitro susceptibility of Finnish *B. pilosicoli* isolated since 2008.
Materials and Methods: Altogether 167 *B. pilosicoli* isolates were obtained from diagnostic samples (porcine faecal samples or intestinal contents) from years 2008- 2015. The samples were submitted to the laboratory by herds that were experiencing diarrhoea problems in growing pigs. Minimum inhibitory concentrations (MIC) for tylosin, lincomycin, tiamulin and valnemulin were tested by VetMICBrachy method.
Results: Decreased susceptibility to tylosin was detected in 101 isolates (60,5 %), (MIC > 2 µg/ml) and to lincomycin in 44 isolates (26,3 %)(MIC > 4 µg/ml). All isolates were sensitive to tiamulin (MIC ≤ 1 µg/ml) and valnemulin (MIC ≤ 1 µg/ml).
Conclusion: The Finnish *Brachyspira pilosicoli* isolates from years 2008- 2015 showed no trend of increased antimicrobial resistance and all the isolates were susceptible to tiamulin and valnemulin. Widespread tylosin resistance and resistance to lincomycin in some isolates indicates that the use of tylosin or lincomycin for treatment of porcine colonic spirochetosis should be based on antimicrobial susceptibility testing of *B. pilosicoli* recovered from pigs with clinical enteritis.

Disclosure of Interest: None Declared

Keywords: antimicrobial resistance, brachyspira

Bacteriology and Bacterial Diseases
BRACHYSPIRA

PO-PF3-190

Genetic diversity and evolution of Brazilian Brachyspira hyodysenteriae isolates

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Introduction: *Brachyspira hyodysenteriae* is the primary etiologic agent of Swine Dysentery. The disease causes severe mucohaemorrhagic diarrhoea, and thereby severe economic losses to swine production. This study aimed to evaluate the genetic diversity, epidemiology and phylogeny among Brazilian isolates of *B. hyodysenteriae* obtained from outbreaks in 1990s and from 2011 to 2015.
Materials and Methods: Forty-eight Brazilian isolates of *B. hyodysenteriae*, from five Brazilian States, Mato Grosso (n=1), Minas Gerais (n=23), Rio Grande do Sul (n=8), Santa Catarina (n=12) and São Paulo (n=4) were isolated and selected between 2011 to 2015 from the bacteria collection of Molecular Pathology Laboratory of Univerdade Federal de Minas Gerais. For a temporal evaluation at Brazilian level, seven *B. hyodysenteriae* isolates obtained in Rio Grande do Sul in 1990s were also used. Seven MLST loci (*adh*, *gdh*, *pgm*, *alp*, *glpK*, *thi* and *est*) were amplified and sequenced. The sequences of housekeeping genes were concatenated in BioEdit and aligned using CLUSTALW. To eliminate poorly aligned sequences, conserved blocks were selected using the software Gblocks and used for phylogenetic analysis. The genetic distance matrix was obtained using Kimura's two-parameter model, and an evolutionary tree was created using the neighbour-joining method with Mega6. Bootstrap values from 1000 replicates were displayed as percentages. The evolutionary model of all isolates was addressed by Tajima's D Neutrality Test using the software DnaSP v5. In addition, the Simpson index was calculated to determine the degree of genetic diversity of Brazilian isolates of *B. hyodysenteriae*.
Results: The 48 strains were grouped into six different clusters. Generally, the classification was related to the periods in which the samples were isolated, with specific variations between the groups. The most significant information was observed in cluster 1, wherein the same clonal type was isolated in seven different farms and three States, without clear epidemiological connections. The cluster 5 was assigned with samples from Rio Grande do Sul/1990's and an isolated from Minas Gerais/2015, showing a recurrence of the old genotypes in the country. The cluster 6 was composed of strains from different states isolated in 2011. The Tajima's Neutrality Test (D= -2.1352, P=0.05) showed a purifying selection for the seven genes evaluated and the Brazilian populations of *B. hyodysenteriae* presented a high genetic diversity (Simpson index = 0.8316).
Conclusion: Brazilian isolates of *B. hyodysenteriae* had a high genetic diversity and samples from different states and different periods of isolation were grouped in the same clusters.

Disclosure of Interest: None Declared

Keywords: MLST, Molecular Epidemiology, Swine Dysentery

Poster Abstracts

Bacteriology and Bacterial Diseases
BRACHYSPIRA

PO-PF3-122

Genetic associations of "Brachyspira hampsonii" from diverse epidemiological origins and their relationships with other swine Brachyspira species

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Introduction: Outbreaks of bloody diarrhea in swine herds in the late 2000s signaled the re-emergence of swine dysentery in the U.S. Diagnostic investigations confirmed the emergence of a novel pathogen "*Brachyspira hampsonii*" with two distinct genetic groups circulating in U.S. swine. It has since been detected in swine and migratory birds in North America and Europe.
Objective: The aim of this study was to develop and implement a multilocus sequence typing (MLST) scheme in order to molecularly characterize "*B. hampsonii*" and to elucidate the diversity, distribution and genetic relationships of this pathogen from diverse epidemiological sources globally, as well as to evaluate the genetic relationships of "*B. hampsonii*" with other *Brachyspira* species.
Materials and Methods: Using our newly developed MLST scheme, we genetically characterized 81 "*B. hampsonii*" isolates originating from diverse epidemiological sources (different farms, swine systems, countries and host species).
Results: A total of 20 genotypes known as sequence types (11 from the U.S. and nine from other countries) were identified. "*B. hampsonii*" exhibited a heterogeneous population structure with microevolution detected locally within swine production systems. Within the U.S., group I was widespread yet less diverse than group II. The clustering patterns displayed the association of genotypes and their country or swine system of origin. Isolates from migratory birds represented distinct yet closely related genotypes when compared with swine origin isolates. The comparative multilocus sequence analysis of 430 isolates representing pathogenic and commensal *Brachyspira* species from 19 countries and 10 host species depicted clustering by bacterial species. It revealed a close clustering of all "*B. hampsonii*" irrespective of their genetic group, thus providing support for both groups to be considered a single species. It also confirmed the close genetic relatedness of "*B. hampsonii*" with commensals *B. murdochii* and *B. innocens*, rather than other pathogens *B. hyodysenteriae* and "*B. suanatina*".
Conclusion: This is the first study to establish a multilocus sequence typing scheme for "*B. hampsonii*", to characterize its genotypes, and to elucidate its population structure. It highlights the role of MLST in routine surveillance/monitoring and the importance of strict biosecurity measures in preventing the spread of pathogens such as "*B. hampsonii*" between swine herds and also potentially between migratory birds and swine. It also suggests that "*B. hampsonii*" represents a diverse but single bacterial species that has close genetic associations with commensal *Brachyspira* species.

Disclosure of Interest: None Declared

Keywords: Brachyspira hampsonii, MLST, Swine Dysentery

Bacteriology and Bacterial Diseases
BRACHYSPIRA

PO-PF3-096

Correlation between detection of enteropathogenic bacteria and health and productive parameters in finishing pigs in Argentina

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Introduction: Enteric infections cause economic losses in pig production. Increases in costs are associated with reduced average daily gain (ADG), increased mortality rate and increased in use of antibiotics. Porcine proliferative enteropathies (PPE), swine dysentery (SD), porcine spirochetosis and salmonellosis are enteric diseases caused by *L. intracellularis* (LI), *B. hyodysenteriae* (BH), *B. pilosicoli* (BP) and *Salmonella enterica* (SE) respectively. The above mentioned infections have been reported world-wide, however in Argentina their impact on productive parameters were not studied. This study analyzes the relationship between enteric infections and health and productive parameters.
Materials and Methods: Eight farms between 80 to 2500 sows were studied. From each, 120 samples of feces of 8, 14, 17, 20 and 24 weeks-old pigs were collected. DNA was extracted (ZR Fecal DNA MiniPrep, Zymo Research, CA, USA) and was analyzed by multiplex PCR assay to identify LI, BH and BP. For SP, pooled samples were cultures using standard methods. Productive, health and biosecurity information were obtained by an *ad-hoc* survey form. Spearman correlation was applied in order to analyze the association of each pathogen with ADG, weight at 70 days, nursery and fattening mortality, biosecurity practices, and herd size and production system.

Results: *Lawsonia intracellularis*, BH and SE were identified in 16.22% (153/943), 1.38% (13/943) and, 6.3% (9/144) of the samples, respectively. All samples were negative to BP. All farms were positive to LI, 2 to BH and 5 to SE. Detection BH was positively associated with mortality rate (r =0.82, p= 0.04) and negatively with ADG (r = -0.8, p= 0.04). The weight of pigs at 70 days showed a negative correlation with nursery and fattening mortality (r=-1.0 p=0.01; r=0.86 p=0.03) respectively. Biosecurity practices showed a negative correlation with weight at 70 days and negative correlation nursery mortality (r=-0.9 p=0.03; r=0.9 p=0.03) respectively. No correlation with SE, LI, number of sows and production system were found.
Conclusion: -In the study, BH affected productive performance and mortality in grower-finisher pigs. Losses were associated with high mortality and low ADG. Herds with good biosecurity practices have a lower risk of infection, mortality rate and higher ADG.
- A high prevalence of LI in Argentinean herds was found, however no statistically association was found between PPE and decreased ADG. It seems that under the conditions of this study, infection with LI might need of cofactors to impact negatively on the growth rate.

Disclosure of Interest: None Declared

Keywords: enteropathogens,, finishing pigs , productive parameters

Bacteriology and Bacterial Diseases
BRACHYSPIRA

PO-PF3-150

In-vitro susceptibility of Brachyspira spp. clinical isolates to tiamulin and narasin

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Introduction: Brachyspira spp. are a group of enteric pathogens prevalent worldwide in most swine rearing geographies. To determine appropriate therapy for Brachyspira disease, minimum inhibitor concentrations (MIC) of antibiotics are a common first step. Narasin is a recently FDA approved ionophore antibiotic to improve growth in swine in the USA. The objective of this study was to determine the MIC of tiamulin and narasin using Brachyspira isolates from clinical cases.
Materials and Methods: Iowa State University, Veterinary Diagnostic Laboratory, used thirty-one (31) isolates of Brachyspira spp: (10 B. hyodysenteriae, 11 B. hampsonii, and 10 B. murdochii) for MIC testing of narasin and tiamulin. These isolates had been collected from 2008 until 2015 and all isolates were from clinical disease herds from North American origin. The isolates were evaluated in an agar dilution MIC antimicrobial testing method using narasin and tiamulin as test material. Four Brachyspira spp. controls were used. The MIC ranges used in this study were 0.015625 – 8.0 µg/ml for tiamulin and 0.0078125 – 1.0 µg/ml for narasin. These ranges were chosen to include the MIC value of all test organisms. There were 2 MIC replicates for each isolate. Diluent and PBS agar dilution controls were included to exclude growth inhibition from the antibiotic diluent used.
Results: The B. hyodysenteriae isolates had a MIC range of 0.03125 – 4 µg/ml for tiamulin and a point dose MIC of 0.015625 µg/ml for narasin. These isolates had a MIC50 of 0.125 µg/ml and a MIC90 of 1.00 µg/ml for tiamulin.
The B. hampsonii isolates had a MIC range of 0.03125 – 8 µg/ml for tiamulin and a point dose MIC of 0.015625 µg/ml for narasin. These isolates had a MIC50 of 0.0625 µg/ml and a MIC90 of 0.125 µg/ml for tiamulin.
The B. murdochii isolates had a MIC range of 0.03125 – 0.25 µg/ml for tiamulin and a point dose 0.015625 µg/ml for narasin. These isolates had a MIC50 of 0.03125 µg/ml and a MIC90 of 0.25 µg/ml for tiamulin.
No inhibition of growth from diluents was observed.
Conclusion: Brachyspira spp. MIC's for tiamulin determined in this study were similar to Clothier et al previous MIC study. Narasin, a new antibiotic for US swine, inhibited the growth of all Brachyspira isolates at the 0.015625 µg/ml MIC level. These data support the continued evaluation of narasin for swine Brachyspiral colitis.

Disclosure of Interest: T. Marsteller Conflict with: employee

Keywords: Brachyspira hyodysenteriae, narasin, tiamulin

Bacteriology and Bacterial Diseases
BRACHYSPIRA

PO-PF3-163

Prevalence of Brachyspira hyodysenteriae and Brachyspira pilosicoli isolated in Spanish pig farms from 2011 to 2015.

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Introduction: *Brachyspira hyodysenteriae* is the aetiologic agent of swine dysentery (SD), an infectious disease that affects growing and fattening pigs causing severe bloody diarrhoea and haemorrhagic colitis. *Brachyspira pilosicoli* causes a non-fatal and non-bloody milder catarrhal colitis named porcine intestinal spirochaetosis (PIS) in young pigs.
Our purpose has been to determine the prevalence of these two spirochaetes in Spanish farms with diarrhoea and clinical signs of SD during the 2011-2015 period.
Materials and Methods: *Farms and samples:* During 2011, a total of 1846 faecal samples were recovered from 203 farms; 1403 samples belonging to 164 farms were analysed in 2012; the following year, 871 faecal samples were tested from 116 pig farms; during 2014, 858 samples were collected from 117 farms; and finally, a total of 647 pig faeces samples were evaluated from 88 farms in 2015. The farms were distributed through all the Spanish territory. *Microbiological and molecular diagnosis:* All the samples were streaked onto CVS (colistin, vancomicin, spectinomicin) selective agar plates. The strains were isolated from those agar plates showing haemolytic activity and with spirochaetes visible under the microscope. The DNA of these strains was extracted and used as template for PCR detection of the putative haemolysin regulatory gene *tlyA* of *B. hyodysenteriae* and the 16S rDNA gene of *B. pilosicoli.*
Results: In 2011, 95 farms were found to be positive to *B. hyodysenteriae* (46.80%). This percentage was lower, 39.02%, in 2012, with a prevalence of 64 positive farms. During 2013, the value increased until 44.83% (52 positive pig farms) and it remained stable in 2014, with 44 farms in which it was possible to detect the bacteria (44.44%). Finally, it has been possible to observe a decrease of the prevalence during 2015, with 31 positive farms, a 35.23%. Otherwise, during this period, the prevalence of *B. pilosicoli* has ranged between 1.22 and 2.56%, not being detected any case in 2013 and 2015. None of the farms was positive to the both pathogenic strains.
Conclusion: Although there are several factors that can affect the appearance of *B. hyodysenteriae* infections such as the management, the housing or the diet, our results indicate that SD is present in a 35.23-46.80% of the studied farms with diarrhoea in adult pigs and clinical suspicion of swine dysentery in Spain. Moreover, the trend to a lower antimicrobial susceptibility among *B. hyodysenteriae* isolates could complicate the control of this infection in swine farms. Conversely, the low incidence of *B. pilosicoli* suggests that PIS plays a minor role in digestive processes in pigs of the Spanish farms analyzed in this study.

Disclosure of Interest: None Declared

Keywords: Brachyspira, Prevalence, Spain

Poster Abstracts

Bacteriology and Bacterial Diseases
BRACHYSPIRA

PO-PF3-124

Antimicrobial susceptibility of 150 isolates of Brachyspira hyodysenteriae recovered from Spanish swine dysentery outbreaks during 2011-2015.

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Introduction: The antibiotic resistance of field strains of Brachyspira hyodysenteriae has increased in the last years in Spain as well as in other countries due to the high use of antibiotics for the control of swine dysentery (SD). For this reason antimicrobial susceptibility tests are becoming essential for an effective control of this disease.
Our objective was to study the antibiotic susceptibility of Spanish field strains of Brachyspira hyodysenteriae in order to provide swine production companies updated data on the most appropriate antibiotic treatment.
Materials and Methods: *Bacterial strains*: 150 field strains of *B. hyodysenteriae* isolated in Spain during 2011 to 2015 were investigated.
Antimicrobial susceptibility test: Each strain was suspended in a commercial plate (VetMIC™ Brachy v2) following the manufacturer's instructions. The plates have six rows, each containing different concentrations of one of the following antibiotics: tiamulin, valnemulin, doxycycline, tylvalosin, tylosin and lincomycin.
The minimum inhibitory concentration (MIC) was determined as the lowest concentration of the antibiotic that prevented visible growth.
The lowest concentrations that completely inhibited the growth of 50% and 90% of the isolates, MIC_{50} and MIC_{90}, respectively, were calculated for each antibiotic.
Results: During the studied period, the value of the MIC_{50} has increased from 1 to 2 µg/ml for tiamulin, from 1 to 4 µg/ml for valnemulin and from 4 to 16 µg/ml for tylvalosin. Regarding to doxycycline and lincomycin, this value has ranged between 0,5-1 µg/ml in the case of doxycycline and between 16-32 µg/ml for the lincomycin. Finally, the value has been >128 µg/ml for the tylosin during the five years.
The values of the MIC_{90} have remained stable in all the antibiotics except tylvalosin, which has increased from 8 to 32 µg/ml.
Conclusion: Along the past five years, the MIC_{50} values for three of the six most relevant antimicrobials used to control SD have increased in the Spanish isolates of *Brachyspira hyodysenteriae*. These results imply that this bacteria is becoming resistant to most commonly available antibiotics used to deal with SD. That fact produces a serious health problem in pigs, increasing the risk of infections among animals, and negative influences on swine production. Careful use of antibiotics and the use of other alternatives such as autovaccines or natural feed additives will minimise the development of more antibiotics resistant strains and provide a possible solution to the global problem of antibiotic resistance.

Disclosure of Interest: None Declared

Keywords: Antibiotics, Brachyspira hyodysenteriae, Resistance

Bacteriology and Bacterial Diseases
CLOSTRIDIA

PO-PF3-147

Clostridium difficile and mesocolon edema syndrome of neonatal pigs: studies of pathogenesis in a gnotobiotic pig model

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Introduction: Mesocolon edema syndrome is recognized as a common cause of diarrhea in pigs less than 2 weeks of age, and has been associated with neonatal colonization by *Clostridium difficile*, *C. perfringens,* or often as a combined infection. In the United States, *C. perfringens* type A is increasing in prevalence, but *C. difficile* colonization and subsequent enterotoxemia is still an important cause of the syndrome. In this study, we directly examined the events of colonization of the lower intestinal tract by *C. difficile* in a gnotobiotic pig model to gain a preliminary understanding of pathogenesis for the syndrome.
Materials and Methods: Two groups, totaling 36 pigs, were delivered by caesarian section into flexible film isolators and associated with a defined normal intestinal flora free of clostridial organisms and other enteric pathogens. Pigs were then assigned to treatment groups, based on variation of timing of antibiotic prophylaxis, antibiotic therapy, probiotic prophylaxis, and challenge with either toxigenic or non-pathogenic *C. difficile*. Evaluation of colonic colonization and lesion development was then pursued through the use of selective culture, genome detection, measurement of host substrate compounds such as bile acids by HPLC, and measurement of host response through tissue cytokine assays, histopathology, and immunohistochemistry.
Results: In this model, colonization by *C. difficile* was easily established and verified by culture and genomic endpoints. Variation of early colonic and mesocolonic lesions was observed between groups, which depended on sequence and nature of antibiotic or probiotic treatments, as well as the timing of challenge with toxigenic *C. difficile*.
Conclusion: Colonization of the lower intestine in this gnotobiotic model was largely dependent on timing and dosage, while lesion initiation appeared to more related to prior manipulation of the microflora through probiotic and antibiotic administration.

Disclosure of Interest: None Declared

Keywords: Clostridium difficile, Mesocolon edema syndrome, Neonatal diarrhoea

Bacteriology and Bacterial Diseases
CLOSTRIDIA

PO-PF3-249

HISTOLOGICAL EFFECTS OF THE CHALLENGE WITH Clostridium difficile IN WEANED PIG

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Introduction: Infection with *C. difficile* may have subclinical signs and light manifestations of disease; its transmission is fecal-oral route, considered as the principal way of contamination. As bacterial infections have negative impact, on the roster, this study has relation with the ways of transmission of *C. difficile* in swine. This study was to evaluate the transmission of *C. difficile* via aerogen using PCR and histological section of tissue.
Materials and Methods: Special isolation booths were use. Six weaned pigs (15 days old) were obtained from a free *C. difficile* farm. Were installed in couples in a safe cabinet and microbiologically monitored daily during 18 days. Cabinets were placed in a straight line (cabinet 1 - control pigs, cabinet 2 - inoculated and cabinet 3 – sentinel pig). This way air flows from the control to the sentinel. The inoculum was *C. difficile* VPI A/B 10463, with 7.21 x 10^8 CFU/mL. An aliquot of 3 mL was administered orally into the pharyngeal region to the pigs of group 2. Control and sentinel pigs received only sterile BHI broth (3 mL) as placebo.Were evaluated for clinical sings of infection, after this period were euthanized and necropsied for lesion evaluation. Liver, spleen, tonsils, mesenteric lymph nodes and ileocolic, jejunum, ileum, proximal and distal colon and cecal contents of the three groups were collected for microbiological and histological analysis. Fragments were placed into BHI, incubated 37°C for 48h in anaerobiosis for clostridial growth. DNA extractions were performed by boiling technique. The PCR reactions were directed for the detection of genes encoding toxins *tcdA* and *tcdB* with annealing temperature of 52°C for 1 min. For histopathological analysis fragments were collected from the same portions and located in bottles with 10% formalin.
Results: PCR technic confirmed *C. difficile* in jejunum and colon of the infected group, meanwhile in control and sentinel groups samples were negative for this pathogen. In the histological analysis control and sentinel group did not presented apparent lesions in selected portions. Piglets of the infected group showed the liver, spleen, tonsils and lymph nodes reactive, besides the spleen that presented lymphoid rarefaction of white pulp, and calcification in interstice of the tonsils and apoptosis with invasion of the lymphocytes epithelium. The intestinal crypts of the mucosa showed hyperplasia of the caliciforms cells and mucus. Among animals of the infected group, enteritis traits and denudation of the villi with reactivity in Pleyer plates were observed.
Conclusion: It was concluded that there was no infection of the sentinel group via aerogen, confirmed by PCR and histologic analysis.

Disclosure of Interest: None Declared

Keywords: anaerobes, swine, Transmission

Bacteriology and Bacterial Diseases
CLOSTRIDIA

PO-PF3-129

In vitro susceptibility of Czech porcine isolates of C. perfringens Type A cpb2+ to amoxicillin, ampicillin and amoxicillin-clavulanic acid

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Introduction: *Clostridium perfringens* type A enteritis is a frequent disease of the new-born piglets with a major economic impact in the Czech Republic and important swine producing countries as well. Different antimicrobials may be used for treatment of piglets affected by clostridial enteritis. There are only a few reports on antimicrobial susceptibility of *C. perfringens* from pigs. Overall, the most common resistance in *C. perfringens* is to tetracyclines . The aim of the study was evaluate sensitivity pattern for aminopenicillins as important group of antibiotics for control of clinical infection in piglets.
Materials and Methods: For the trial purposes 56 isolates of *C. perfringens cpb2-* positive from clinical cases of neonatal enteritis from period 2007 - 2015 from farm in Czech Republic were chosen. Each isolate represented one piglet (1 – 14 days) and one different farm per year. Identification of *C. perfringens* was performed based on the demonstration of the characteristic morphology after 24-48 hour anaerobic cultivation on Wilkins-Chalgren agar (WCA). Definitive identification was performed by Matrix-Assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS), (Bruker Daltonics, Germany). Genetic characterization of isolates was finalized by the determination of species-specific gene (*cpa*) and *cpb2* by specific PCR . MIC testing was performed according to CLSI guidelines by agar dilution method (CLSI, 2013). Data in the final report are presented as MIC range and MIC_{50}, MIC_{90}.
Results: Range of MICs to ampicillin in 56 *C. perfringens* Type A *cpb2+* isolates was from <0, 01 to 4 mg/l, MIC_{50} was 0,125 mg/l and MIC_{90} 2 mg/l.
Range of MICs for amoxicillin was same like in case of ampicillin (from <0, 01 to 4 mg/l), but MIC_{50} achieved 3 times lower value: 0, 03 mg/l, MIC_{90} for amoxicillin was 2 mg/l.
Range of MICs values to amoxicillin-clavulanate was established from <0, 01 to 8 mg/l, MIC_{50} was <0, 01 and MIC_{90} was 2 mg/l.
Conclusion: Based on results we can conclude that aminopenicillins are very good option for the treatment of *C. perfringens* type A infection of piglets. According currently available interpretive criteria we haven't found isolates resistant to aminopenicillins. We have confirmed that prevalence of strains producing beta- lactamases is very low. According rational use of antimicrobials we consider amoxicillin as optimal choice for treatment clinical cases. Major differences between amoxicillin and ampicillin were not observed but amoxicillin showed better MIC_{50} parameter and distribution of MICs.

Disclosure of Interest: None Declared

Keywords: antimicrobial resistance, Clostridium perfringens

Poster Abstracts

Bacteriology and Bacterial Diseases
CLOSTRIDIA

PO-PC02-008

The role of oral toltrazuril and iron combination in the weaning weight gain versus conventional coccidiosis and anaemia control in neonatal piglets

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Introduction: *Cystoisospora* (syn. *Isospora*) *suis* is a leading cause of diarrheal disease in neonatal piglets and the disease is related to considerable economic losses. The metaphylactic treatment with toltrazuril 3 to 5 days after birth is a common control measure practiced in many countries. Another essential practice is the prevention of neonatal iron deficiency anaemia as piglets are born with small iron reserves (35 to 50 mg) and sow milk contains only limited quantities of iron. Iron supplementation is commonly administered to piglets in the first days of life by intramuscular injection of iron dextran. An oral combination of toltrazuril and iron dextran was developed with the objective to reduce the total number of interventions, to improve animal welfare and to promote labour-saving gain for the farmer, and product effectiveness in the control of coccidiosis and iron deficiency anaemia is demonstrated.
Materials and Methods: The field evaluation was run in a commercial pig farm located in Brazil, Minas Gerais State, with history of coccidiosis. Just after birth piglets were individually weighted and ear tagged. On the third day of life the 144 piglets allocated to the control group (CG) each received 1 mL Baycox®, containing 50mg/mL toltrazuril orally and commercially available iron (200 mg/piglet) by intramuscular injection. The 148 piglets allocated to the treatment group (TG) each received 1 mL toltrazuril and iron combination orally (Baycox® Iron, Bayer) containing 50 mg/mL toltrazuril and 228 mg iron as iron dextran. The individual weaning weight of the piglets was recorded (21 days of life) and during the study the animals were observed daily – feces aspect, paleness and diarrhea signals. The parameters evaluated were weaning weight gain and paleness of piglets during suckling period.
Results: The mean piglet body weight at birth was 1.419 kg for the TG piglets and 1.448 kg for the CG piglets. At weaning – 21 days of life – piglet body weight for the TG was 5.940 kg and for CG was 6.063 kg. Analysis of variance showed no significant difference between treatment group body weights at birth and at weaning ($p<0.05$). No differences were observed between treatment groups regarding diarrhea signals and pale animals.
Conclusion: Baycox Iron oral formulation was effective in the maintenance of body weight gain during suckling period, providing coccidiosis and iron deficiency control when compared to the conventional practice. Thus, provides labor-saving and reduces stressful intervention to the piglets.

Disclosure of Interest: E. Dantas Conflict with: Bayer employee, K. Streyl Conflict with: Bayer employee, C. Bhushan Conflict with: Bayer employee

Keywords: Coccidiosis, Iron-deficiency, Toltrazuril

Bacteriology and Bacterial Diseases
CLOSTRIDIA

PO-PF3-206

Comparison Of The Development Of Antibodies Against The A And B2 Toxins After Vaccination Of Sows With A Clostridium Perfringens Type A Toxoid Vaccine

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Introduction: There are two registered vaccines available (Clostriporc A, Enteroporc A, IDT Biologika GmbH) to prevent suckling piglet diarrhea caused by *Clostridium perfringens* type A (CpA). These are based on the α- and β2-toxoids. The goal of this study was to evaluate the development of antibodies against the α- and β2-toxins after recurrent vaccination of gilts with ENTEROPORC A under laboratory conditions.
Materials and Methods: The gilts were vaccinated *i.m.* twice (5 and 2 weeks) before the first parturition and once two weeks before the second parturition. A control group was vaccinated at the same points in time with a saline solution. Sera were taken from the sows before the first (B0), second (B1) and third (B3) vaccination as well as sera and colostrum at the first (B2) and second parturition (B4). These samples were analyzed for antibodies against the α-toxin (ELISA, Lecithovitellin-neutralization test) and β2- toxin (ELISA). Additionally, suckling piglets from the first litter of the vaccinated sows and the control sows were inoculated *i.p.* with a sterile filtered supernatant of a heterologous CpA strain.
Results: All twofold and threefold vaccinated sows showed a significant (Mann Whitney U test, one tailed, $p<0.05$) increase of antibodies against the α- and β2-toxins in the serum at parturition (B2 and B4) and in the colostrum. Piglets from sows vaccinated twice were protected during the toxin challenge. The third vaccination resulted in comparison with the second vaccination in a further significant increase ($p < 0.05$) of antibodies against α- and β2-toxins in serum (figure 1 and 3) and colostrum (figure 2 and 4).
Conclusion: The results show that the third vaccination resulted in a further significant increase of antibodies in the colostrum compared to the basic vaccination. These results are especially interesting when considering the problem of providing colostrum to an increasing number of piglets born to hyperprolific sows. Further studies have to show whether a double vaccination of gilts before insemination and a third vaccination before the first parturition have the same effect.

Disclosure of Interest: J. Finzel Conflict with: IDT Biologika, G. Hagemann Conflict with: IDT Biologika, V. Florian Conflict with: IDT Biologika, V. Gotter Conflict with: IDT Biologika, S. Springer Conflict with: IDT Biologika

Keywords: immunity, Suckling piglet diarrhea, Vaccination

Bacteriology and Bacterial Diseases
CLOSTRIDIA

PO-PC02-006

Supplementation of sow diets with a Saccharomyces cerevisiae fermentation product on lactation performance and fecal Clostridium perfringens

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Introduction: *Clostridium perfringens* is a gram-positive bacteria that can cause diarrhea and is commonly transferred from the sow to the piglet. The objective of this study was to evaluate dietary addition of a *Saccharomyces cerevisiae* fermentation product during late gestation through a 21 d lactation on sow and litter performance, milk components, and fecal *Clostridium perfringens*.
Materials and Methods: On d 93 of gestation, sows (n = 41; PIC 29) were blocked by parity and BW and assigned to 1 of 2 dietary treatments: control (CON) or CON + 0.20% *Saccharomyces cerevisiae* fermentation product (XPC; Original XPC™, Diamond V, Cedar Rapids, IA). Control gestation (3315 kcal/kg of ME; 0.60 % SID Lys), and lactation (3.30 kcal/kg of ME; 1.04 % SID Lys) experimental diets were formulated to be devoid of antibiotics and to meet or exceed NRC 2012 recommendations. Colostrum (within 6 h of partum) and milk (d 14) samples were collected from individual sows to determine IgG, IgA and lysozyme concentration. In addition, fresh grab fecal samples were obtained from sows and their litter on d 7 postpartum to quantify *Clostridium perfringens* using PCR.
Results: Sows fed XPC had heavier 110 d BW (270.8 vs. 266.4 kg; $P = 0.05$) and gained more weight (22.85 vs. 18.48 kg; $P = 0.05$) during late gestation than CON. Sows fed XPC tended to have higher ADFI during lactation week 1 (3.64 vs 3.19 kg/d; $P = 0.07$) and overall (5.84 vs. 5.37 kg/d; $P = 0.10$). Moreover, XPC-fed sows had heavier piglet birth weights (1.40 vs. 1.28 kg; $P = 0.05$), reduced numbers of stillborn pigs (1.13 vs. 1.88; $P = 0.04$), and heavier average piglet BW on d 7 postpartum (2.86 vs. 2.60 kg; $P = 0.04$) than those fed CON diet. Colostrum IgA (14.34 vs. 15.06 mg/mL), IgG (80.93 vs. 86.80 mg/mL) and lysozyme (81.52 vs. 108.33 U/mL) were similar between XPC- and control-fed sows ($P > 0.35$). Similarly, milk IgA did not differ between treatments ($P > 0.79$). Milk lysozyme tended to be lower in sows fed XPC (23.6 vs. 31.6 U/mL; $P = 0.09$) when compared to CON-fed sows. Finally, XPC-fed sows had reduced levels of *Clostridium perfringens* in both sow (2.86 vs. 3.33 log CFU; $P = 0.09$) and piglet (5.09 vs. 5.39 log CFU; $P = 0.02$) feces compared to those fed CON diet.

Conclusion: Supplementing XPC to sows in late gestation and lactation improved sow and litter performance, modulated milk lysosome level, and reduced the level of fecal *Clostridium perfringens* in both the sow and piglets.

Disclosure of Interest: None Declared

Keywords: Clostridium perfringens, pig, Saccharomyces cerevisiae fermentation product

Bacteriology and Bacterial Diseases
E.COLI

PO-PF3-273

Field study to assess the economic benefit of vaccination with SUISENG® on a Portuguese farm.

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Introduction: The purpose of this study was to demonstrate the economic benefit and improved yield of groups of gilts vaccinated with SUISENG®, on a farm with a high prevalence of neonatal diarrhoea caused by *E.coli/Clostridium*.
Materials and Methods: 49 clinically healthy, PRRSV-positive and ADV-negative gilts were selected on a farm with 800 sows, and divided into 4 groups, 2 vaccinated (V1 n=13; V2 n=12) and 2 not vaccinated (NV1 n=12; NV2 n=12) in alternating chronological order. The recommended vaccination protocol for SUISENG® was used for the vaccinated group.
In order to assess the economic benefit of vaccination with SUISENG®, the number of piglets born alive and the number of animals weaned at the end of lactation were recorded by group and by sow; also recorded were the mortality rate during lactation and the weights of all the piglets at birth and at weaning, thus giving the ADG (average daily gains), by group and by animal.
The animals which received antibiotic treatment because of diarrhoea, and the cost of this, were also recorded. From all this, the economic benefit of vaccination with SUISENG® was assessed on the basis of the decrease in mortality rate, increased ADG, treatments given and the cost of vaccination.
Results: With regard to piglets born alive, no statistically significant differences were recorded; with regard to the number of weaned piglets, there were statistically significant differences between the vaccinated group of 268 piglets and the non-vaccinated group of 139 piglets, with mortality rates of 4.3% (12 piglets - caused by crushing and handling) and 47.5% (126 piglets - all the piglets showed clinical signs of diarrhoea at necropsy) respectively.
With regard to average weights, no statistically significant differences were recorded at birth, but were recorded at weaning (27 days) with 7.809 kg for the vaccinated group compared to 7.385 kg for the non-vaccinated group, with ADG of 250 g and 202 g respectively.
In addition, the non-vaccinated group were affected with diarrhoea and all the piglets were treated with amoxicillin+colistin i.e. an increase of 0.33 Euros per weaned piglet. In contrast, litters from the vaccinated groups did not suffer from diarrhoea and there was merely an increase in the production cost of 0.27 Euros per weaned piglet as a result of sow vaccination with SUISENG®.
Conclusion: The economic benefit of vaccination with SUISENG® was demonstrated, not only because of the cost of losses caused by neonatal diarrhoea, but also because of the increased cost resulting from antibiotic treatment of animals from non-vaccinated groups. The cost at the end of lactation was therefore reduced and the efficacy of vaccination with SUISENG® was demonstrated.

Disclosure of Interest: None Declared

Keywords: Economic benefit, Neonatal diarrhoea, Vaccination

Poster Abstracts

Bacteriology and Bacterial Diseases
E.COLI

PO-PF3-213

Preventative use of Lactobacillus acidophilus fermentation product on postwean K88 and salmonella: Quantitative assessment of prevalence and severity

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Introduction: This case study examines a herd with a history of endemic K88 *E. coli* and Salmonella diarrhea post wean. Signs included acutely: diarrhea and dehydration; subacutely: acute signs plus gradual fat loss; and chronically: muscle loss and rough hair coat. Control is sporadic with both vaccination and medication attempts. Currently Neomycin therapy is used. In an effort to increase treatment efficacy, reduce dependence on antibiotics, and support health and performance of nursery pigs, a *Lactobacillus acidophilus* fermentation product (LAFP, SynGenX®; Diamond V, Cedar Rapids, IA) was examined as preventative support to current control methods prior to typical onset of diarrhea. This product has been shown to alter enteric bacteria concentrations and the immune response; and therefore may assist pigs in coping with both *E. coli* and Salmonella before they cause disease.
Materials and Methods: Investigators monitored the impact of LAFP (1kg per MT) and Neomycin (22 mg/kg) in the water (1 week) on prevalence and severity of clinical signs compared to pigs receiving Neomycin alone. Five barns were enrolled. Severity was divided into the following categories: acute, subacute, chronic, or euthanize/cull. Clinical signs of Fallout, Respiratory, Diarrhea, Lameness, Neurologic, and Other were measured each week for both prevalence and severity of respective signs. Death loss, stools per pen, and injectable treatments were measured. Statistical analysis was performed using Fisher's Exact test for proportions using MedCalc 15.11 (MedCalc Software, Ostend, Belgium).
Results: Death loss on the treatment side was 56% less than the control (P=0.003). Number of necessary individual treatments was 40% less than control (P<0.0001). Total fallouts were reduced at two weeks post entry when diarrhea is typically most severe. At 2 weeks post entry, acute fallouts were reduced post entry in 3/5 barns (B3NE P=0.016, B3NW P<0.0001, B4NE P=0.031); subacute fallouts were reduced in 3/5 barns (B1N P=0.011, B3NW P<0.001, B4NW P=0.041); chronic fallouts were reduced at 2 weeks post entry in 1/5 barns (B3NW P=0.002). Stools per pen were reduced in barns experiencing diarrhea at 2 weeks post entry (B3NE P<0.001, B3NW P<0.001, and B4SW P=0.004) and at 5 weeks post entry (B1N P<0.001, B2NE P=0.009, B3NW P=0.035).
Conclusion: Preventative therapy with LAFP reduced death loss, number of pigs that needed individual treatment, reduced fallouts, and diarrhea in pigs. Additional studies for replacing antibiotic therapy completely are warranted. In this case, producer achieved his goal of using less antibiotic due to reduced injectable therapy needed in treatment groups.

Disclosure of Interest: None Declared

Keywords: E.coli, Lactobacillus acidophilus fermentation product , Salmonella

Bacteriology and Bacterial Diseases
E.COLI

PO-PF3-189

Therapeutic use of Lactobacillus acidophilus fermentation product on post-wean K88 and salmonella: Quantitative assessment of prevalence and severity

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Introduction: This case study examines a herd with a history of endemic K88 *E. coli* and Salmonella diarrhea post wean. Signs included acutely: diarrhea and dehydration; subacutely: acute signs plus gradual fat loss; and chronically: muscle loss and rough hair coat. Control is sporadic with both vaccination and medication attempts. Currently Neomycin therapy is used. In an effort to increase treatment efficacy, reduce dependence on antibiotics, and support health and performance of pigs, a *Lactobacillus acidophilus* fermentation product (LAFP, SynGenX®; Diamond V, Cedar Rapids, IA) was examined as support to current therapy (implemented after diarrhea started in herd). This product has been shown to alter enteric bacteria concentrations and immune response; and therefore may assist pigs in coping with both *E. coli* and Salmonella before they cause disease.
Materials and Methods: Pigs were not treated with Neomycin or LAFP for 2 weeks post entry until diarrhea was established in groups. At 2 weeks post entry, a 36% diarrhea prevalence was observed on treatment side and a 40% diarrhea prevalence was observed on control side. K88 *E.coli* and Salmonella were isolated from a pig in treatment group and from a pig in control group in the same barn. Neomycin (22 mg/kg for 1 week) was administered to both groups. LAFP at 1kg per MT fed continuously was included in feed on treatment side of the barn. Quantitative assessments of clinical signs were measured each week for both prevalence and severity in subpopulation. Severity was divided into the following categories: acute, subacute, chronic, or euthanize/cull. Additionally, death loss, stools per pen, and injectable treatments were measured.
Results: After LAFP implementation, a fallout reduction trend as early as 1 week post administration occurred with 33% fallout on control side and 21% fallouts on treatment side. Significant reduction (P<0.001) in total fallouts occurred 3 weeks post administration with 11% fallouts on control side and 1% fallouts on treatment side. One week post implementation of LAFP a significant reduction in amount of chronic diarrhea (P=0.012) and a significant reduction in amount of diarrhea stools per pen (P=0.009) occurred.
Conclusion: Regular quantitative assessment of prevalence and severity of symptoms is essential to understanding impact of therapy. Use showed reduced fallouts and reduced diarrhea in pigs who received LAFP with antibiotic therapy. Clinically, in the therapy groups, the reduction of signs was visually apparent on the treatment side vs the control. Additional studies for replacing antibiotic therapy completely are warranted.

Disclosure of Interest: S. Probst Miller, DVM Conflict with: Diamond V, Conflict with: AgCreate Solutions, Inc, A. Ramirez, DVM, MPH, PhD Conflict with: iowa State, B. Bass, PhD Conflict with: Diamond V, J. Frank, PhD Conflict with: Diamond V

Keywords: E.coli, Lactobacillus acidophilus fermentation product, Salmonella

Bacteriology and Bacterial Diseases
E.COLI

PO-PF3-231

Probiotic treatment of an infection with Shiga toxin-producing Escherichia coli O104:H4 in a gnotobiotic piglet model

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Introduction: In 2011 several European countries were hit by an outbreak with Shiga toxin-producing *E. coli* (STEC) O104:H4. More than 20 % of the 3842 diseased people developed haemolytic-uraemic syndrome. The origin of this emerging pathogen is an enteroaggregative *E. coli*, which acquired a phage-encoded Stx2-gene. To date, no causative treatment for disease caused by STEC is available. Hence, suitable *in vivo* models are important for studying STEC and for testing new treatment approaches.
Using gnotobiotic piglets as a model to reproduce infection with human pathogenic STEC O157:H7 are well described. We modified the model using the 2011 *E. coli* O104:H4 outbreak strain. Additionally, effect of metaphylactic treatment of *E. coli* O104:H4-infection with *E. coli* G3/10, a component of the probiotic Symbioflor2, was evaluated. *E. coli* G3/10 produces among others a potent microcin and is a potential candidate for the treatment of infections with diarrheagenic *E .coli*.
Materials and Methods: Gnotobiotic piglets were surgically delivered and assigned to 5 groups: I) negative control, II) infection with STEC O157:H7, III) infection with STEC O104:H4, IV) infection with STEC O104:H4 and treatment with *E. coli* G3/10, V) treatment with *E. coli* G3/10. Animals were infected orally on day of birth (0), and treated on days 0-7, if applicable. Neurological status, hydration status, appetite and general behaviour were assessed every 4 hours to determine clinical score. Blood chemistry, total blood count, urinalysis and bacteriological examination of faeces were performed. Lymphnode-cells and PBMCs were isolated, labelled and analysed by flow cytometry. Euthanasia was performed on days 8-12 or earlier to avoid suffering.
Results: For statistical analysis of clinical data Mann-Whitney-U-test was performed. Significant differences were found for clinical scores between group I and all groups mono- or coinfected with pathogenic *E .coli* (II, III, IV). There was no significant difference between negative control (I) and treatment group (V). Coinfection with *E .coli* G3/10 and STEC O104:H4 (IV) resulted in lower clinical scores in comparison to monoinfection with STEC O104:H4 (II), but with no significant difference. Severe neurological signs appeared only in group III. In group IV lack of appetite and dehydration mainly contributed to the clinical score.
Conclusion: Analysis of clinical score suggests, that *E. coli* O104:H4 may cause disease in gnotobiotic piglets. Animals coinfected with pathogenic and probiotic *E. coli* (IV) performed better than monoinfected animals (II), but with no significant differences. For conclusive interpretation of data, results of histological and bacteriological examination have to be taken into account.

Disclosure of Interest: B. Woechtl: None Declared, K.-H. Waldmann: None Declared, A. von Altrock: None Declared, W. Gerner: None Declared, A. Saalmueller: None Declared, F. Gunzer: None Declared, K. Zimmermann Conflict with: industry partner, M. Koch: None Declared, I. Hennig-Pauka: None Declared

Keywords: E. coli G3/10, E. coli O104:H4, gnotobiotic piglet

Bacteriology and Bacterial Diseases
E.COLI

PO-PF3-296

Antimicrobial resistant E. coli from finishing pigs in non-antibiotic used farms in Thailand: prevalence, phenotypic and genotypic characteristics

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Introduction: In pig production, the animal carriers have been claimed as one of important source for multidrug resistant (MDR) bacteria that are naturally induced beneath a routine antibiotic use for quite a while. At the present, bacterial wild types have been believed that they are adapted from previous managements in the past. This may be resulted from either remaining persistent MDR or becoming resistant wild type strains. Since *Escherichia coli* is the well adaptive bacteria in enteric tract from birth to slaughtering, it can be MDR indicator during production. The objective was to determine antimicrobial resistant situation of *E. coli* isolated from healthy finishing pigs in non-antibiotic used in Thailand by prevalence, phenotypes, and genotypic resistances.
Materials and Methods: A total of 137 *E. coli* were isolated from late finishing pigs from the farms at north part and central area of Thailand where did not used an antibiotic for at least 5 years. The isolates were determined the antibiograms to 19 antimicrobial agents and ESBLs phenotype by an automated test machine (Vitek2, Biomerieux, France). The 18 resistant genes encoding 8 groups of antibiotic and 17 major replicon types belonging to *Enterobacteriaceae* were detected by their approved multiplex and simplex PCR.
Results: By phenotypic profiles, among susceptible *E. coli* to gentamicin, tobramycin, enrofloxacin, marbofloxacin, chloramphenicol and sulfamethoxazole/trimethoprim were found at up to 60%. This result revealed a lower resistant prevalence than those of previous reports derived from antibiotic used farms in Thailand. However, almost of *E. coli* isolates still resisted to ampicillin, amoxiclin, piperacillin and tetracyclines, which conformed to bla_{TEM} and *tetA* gene positive detection. These might imply that the strains resistant to ampicillin, piperacillin and tetracyclines, became a local wild type in the area of study. Regarding to phenotype and genotype, the prevalence of tetracycline resistance in our study was not different from antibiotic used farm. By replicon typing, replicons; F (70.1%) were the most common followed by FIB (56.9%) and I1-Iγ (40.1%), respectively, while, the other replicons were detected at the lower rate (<30%).
Conclusion: The prevalence of *E. coli* resistant to gentamicin, tobramycin, enrofloxacin, marbofloxacin, chloramphenicol and sulfamethoxazole/trimethoprim was remarkably low in the area of antibiotic free, but the isolates still maintained the resistance to piperacillin, amoxicillin, ampicillin and tetracyclines in our area study.

Disclosure of Interest: None Declared

Keywords: antimicrobial resistance, Escherichia coli, finishing pigs, non-antibiotic use, Thailand

Poster Abstracts

Bacteriology and Bacterial Diseases
E.COLI

PO-PF3-300

Prevalence of virulence genes and haemolytic activity in Escherichia coli associated with diarrhoea in grower pigs

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Introduction: Enteric diseases are the most common reason for antibiotic treatment in nursery pigs in Denmark. *Escherichia coli* (*E. coli*) harbouring F4 or F18 adhesins are among the most important causes of post weaning diarrhoea in week one and week two after weaning. *E. coli* is also found as cause of diarrhoea outbreaks in grower pigs in week 3-10 after weaning which accounts for the majority of antibiotic treatments. The objective of the current study was to investigate the prevalence of virulence genes in *E. coli* from outbreaks of diarrhoea in growers and assess the value of using haemolytic activity as a predictor of virulence.
Materials and Methods: Faecal samples from non-treated diarrhoeic pigs two to four week post weaning were collected from three commercial nursery facilities in the eastern part of Denmark. The samples were cultured on blood agar and *E. coli* isolates were analysed for haemolytic activity and by PCR for adhesion factor; (F4, F5, F6, F18, F41), and toxin genes; (VT2e, STa, STb and LT).
Results: A total of 208 *E. coli* isolates from the faeces of 86 pigs were included in this study. The only adhesion factor found were F18 (F18+) which occurred in 46 isolates (22 %). Out of the 46 F18+ isolates 41 (89 %) were positive for toxins and thereby classified as virulent. The combination of toxins found in the 41 F18+ isolates was 22 (53 %) STb+Lt, 10 (24 %) STb and 9 (22%) STa+STb. Virulent F18+ isolates was cultured from the faeces of 22 (26 %) of the 86 diarrhoeic pigs. Of the 208 isolates 54 (26 %) showed haemolytic activity. By using haemolytic activity as marker for F18+ isolates with virulence genes a sensitivity (SE) of 97.6% (CL95% = 87.1%>99.9%), specificity (SP) of 91.6% (CL95% = 86.3%>95.3%), positive predictive value (PPV) of 74% (CL95% = 60.35%>85.04%) and a negative predictive value (NPV) of 99% (CL95% = 96.44%>99.98%) were obtained.
Conclusion: The only adhesin found in this study was F18. Virulence genes were present in 89 % of the F18 + isolates. In approx. ¼ of the diarrheic pigs in this study virulent *E. coli* F18+ were isolated, which indicates virulent *E. coli* F18+ are an important cause of diarrhoea in pigs two to four weeks post weaning pigs in the three study herds. Haemolytic activity was a useful marker for isolates containing both adhesions and virulence genes. The PPV for haemolytic activity as virulence marker was 74 %, which indicates that false positive or presence of other virulence genes than those examined should be considered when using haemolytic activity of *E. coli* as indicator of virulence.

Disclosure of Interest: None Declared

Keywords: diarrhoea, E. coli, nursery pigs

Bacteriology and Bacterial Diseases
E.COLI

PO-PC02-018

Analysis of E. coli isolates found in 'STEC Check' submissions in The Netherlands in 2015

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Introduction: Edema disease (ED) is caused by strains of *Escherichia (E.) coli* that produce shiga toxin 2e (*Stx2e*, STEC). Ecoporc SHIGA is a genetic recombinant vaccine against ED. This vaccine can be given to piglets from the 3rd day of life to induce active immunity against *Stx2e*. However, before the decision to vaccinate is made, the clinical diagnosis of ED on farm should be confirmed in the laboratory. In order to facilitate the laboratory diagnosis of ED, IDT offers the so called STEC Check.
Materials and Methods: For the STEC Check, 15 individual rectal fecal samples are pooled into three pool samples. The analysis is performed at the Institute of Hygiene and Infectious Diseases of Animals, Justus-Liebig-University, Gießen, Germany. First the pools are cultured for the presence of *E. coli*. On average, 6 *E. coli* isolates per pool are characterized by appearance of hemolysis and by multiplex PCR for toxin genes LT-I, ST-IP, ST-II and Stx2e as well as the adhesion factors F4, F5, F6, F18, F41 and intimin.
Results: 676 isolates from 117 samples from 29 submissions were part of this analysis. The submissions were received from April to December of 2015. In 131 of all isolates (19.4%) *Stx2e* was found. In 104 of these 131 isolates *Stx2e* and F18 fimbriae were detected. In 6 of these 104 isolates additionally the SP-II gene was detected. In 44 other isolates of these 104 isolates, both SP-IP and SP-II genes were detected. All 104 Stx2e and F18 gene posititve isoloates were hemolytic E.Coli. 27 *Stx2e* positive isolates lacked the gene for F18 fimbriae. Of these 27 isolates, 15 were hemolytic and 12 were not. Only one of these 27 isolates had another attaching factor, which was intimin. This was a hemolytic strain. Out of 117 samples, 36 had at least one *Stx2e* and F18 positive isolate. In six samples all isolates were *Stx2e* and F18 positive. In more than 83% of all samples other types of *E. coli* isolates besides STEC were also found. Of 29 submissions, 16 (55.2%) had at least one *STEC* isolate with a maximum of 24 positive isolates per submission. In only one submission only one STEC was found.
Conclusion: The decision to vaccinate piglets with Ecoporc SHIGA should be made only after the detection of Stx2e in the herd. In little over half of the STEC Check submissions in the Netherlands in 2015, multiple STEC could actually be shown. In these cases vaccination with Ecoporc SHIGA is advisable. However, during sample analysis, it is necessary to test multiple isolates per sample, because different *E. coli* strains can be present at the same time in the same sample, which could lead to a false negative result, if fewer isolates are analyzed.

Disclosure of Interest: None Declared

Keywords: E. coli STEC, Edema Disease, shiga toxin

Bacteriology and Bacterial Diseases
E.COLI

PO-PF3-101

Antimicrobial resistance of pathogenic Escherichia coli isolated from pigs in Poland

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Introduction: The susceptibility pattern of *Escherichia* (*E.*) *coli* in showed high frequency of resistance in commensal strains. Though commensal indicator organisms are targeted for monitoring of resistance, pathogenic *E. coli* is of major importance for veterinary clinicians. It also reveals important information for resistance surveys. The study aim was to assess the prevalence of antimicrobial resistance of pathogenic *E. coli* isolated from pigs in Poland.
Materials and Methods: A total of 190 pathogenic *E. coli* strains (F4, F5, F18, Stx2e) isolated from 2011 to 2015 from diseased, diarrheic pigs (neonatal, post-weaning) and edema disease were considered. A single isolate of *E. coli* from the same farm was included. The strains were analyzed for their resistance to 13 antimicrobials using the microbroth dilution method (TREK D. S.). The obtained MIC (minimal inhibitory concentration) values were evaluated according to the EUCAST criteria (epidemiological cut-offs) with the exception of tiamulin and tylosin (no EUCAST criteria). PCR targeting *mcr-1* gene was used in colistin resistant isolates.
Results: Resistance was found to all tested antimicrobials, reaching the highest values for oxytetracycline (71.6%), ampicillin (54.2%), trimethoprim/sulphamethoxazole (40.5%), enrofloxacin (38.4%) and spectinomycin (37.4%). Ceftiofur, amoxicillin/clavulanic acid and neomycin resistances ranged between 8.4% and 15.8%.The lowest resistance was observed to colistin (4.7%), florfenicol (5.3%), and gentamicin (5.8%). Among 77 ETEC/STEC strains (Stx2e, edema disease), the resistance was as follows: oxytetracycline (74%), ampicillin (45.4%), trimethoprim/sulphamethoxazole (32.5%), enrofloxacin (29.9%), spectinomycin (28.6%), amoxicillin/clavulanic acid (13.0%), neomycin (10.4%), colistin (6.5%), ceftiofur (3.9%), gentamicin (3.9%), and florfenicol (2.6%). All tested isolates had tylosin MIC=64 µg/mL. About 95% of isolates had tiamulin MIC > 16 µg/mL. Single *E. coli* (F4+) carried *mcr-1* gene.

Conclusion: The results of our study confirmed the highest *in vitro* sensitivity of pathogenic *E.coli* isolated from pigs to colistin, florfenicol and gentamicin. Besides high level of resistance to oxytetracycline, ampicillin, trimethoprim/sulphamethoxazole and spectinomycin, our results also showed high level of resistance to enrofloxacin, which is commonly used for treatment. ETEC/STEC strains had similar trends of the resistance as ETEC strains. The high MIC values for tylosin and tiamulin, and plasmid-mediated colistin resistance, might suggest their limited use in treatment or metaphylaxis of swine colibacillosis.

Disclosure of Interest: None Declared

Keywords: antimicrobials, Escherichia coli, MIC

Bacteriology and Bacterial Diseases
E.COLI

PO-PF3-251

Comparison of post weaning zinc oxide and colistin treatment in weaned piglets

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Introduction: The aim of this study was to compare the effect of post weaning treatment of piglets with zink oxide (ZnO) and colistin on health, production and *Escherichia coli* shedding.
Materials and Methods: During two successive weaning rounds, a randomized control study was performed on three commercial pig herds. In each herd, four groups of weaners were created that either received colistin in the feed (CF) (Promycine® 400 IU/mg, premix, VMD, Belgium) or the drinking water (CW) (Colistine Eurovet® 400000 IE/ml, Eurovet, Belgium) or ZnO (Gutal®, 2500 ppm, Huvepharma, Belgium) in the feed (ZnO) during the first two weeks post weaning or remained untreated (NC). Daily weight gain (DWG) was recorded by weighing the pigs at weaning, day 14 and at the end of the nursery period. Also feed intake (to calculate the FCR), mortality and antimicrobial use were recorded. Fecal samples, taken at day 7 and 14 of the nursery period, were tested for the presence of hemolytic *E. coli* as well as the number of total *E. coli.* Dirty backhands were scored every week as a measure for diarrhea.
Results: The piglets from the ZnO group showed a higher DWG during the first two weeks of the nursery period compared to the other groups ($P<0.05$). For the total nursery period, the ZnO group showed numerically the highest DWG, but there was no significant difference compared to the other groups. The feed intake was higher for the ZnO group compared to the CF and CW group ($P<0.05$). The FCR and the percentage of mortality did not differ significantly between the four treatment groups. Total *E. coli* count was lower for the CW group compared to the ZnO and the NC group ($P<0.05$). The NC group had the highest percentage (73.5%) of positive samples for hemolytic *E. coli* ($P<0.05$). The ZnO group showed the lowest percentage of dirty backhands ($P<0.05$).
Conclusion: In this study, ZnO (Gutal®) showed to be as effective as colistin on health and production parameters, with a better DWG during the supplemented period and a reduced diarrhea score.

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Keywords: Colistin, E. coli diarrhea, zinc oxide

Poster Abstracts

Bacteriology and Bacterial Diseases
E.COLI

PO-PF3-269

Observations regarding growth and uniformity of weaned piglets after vaccination with Ecoporc SHIGA®

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Introduction: Edema disease (ED) is caused by Shiga toxin 2e (*Stx2e*) producing *E.coli* (STEC). ED can cause substantial losses in weaned pigs. Ecoporc SHIGA® is a genetic recombinant vaccine against ED made by IDT Biologika GmbH. The vaccine is used in piglets from the 4th day of life to induce active immunity against *Stx2e*. The aim of this study was to see whether there is a difference in daily weight gain after vaccination with Ecoporc SHIGA® compared to an unvaccinated control group. Vaccination was started based on clinical signs and after confirmation of the presence of STEC in fecal samples.
Materials and Methods: On this farm, at weaning all piglets are placed randomly in compartments. A control group (c) of unvaccinated animals (n=206) and a vaccinated group (v) (n=242) of consecutive weekly batches housed in two different compartments, were weighed individually. Besides the vaccination, the animals were kept under the same circumstances. The feed, the medication schedule and the weaning age (23 days) were the same. Weaning weight (c =6 kg, v = 5 kg) was a little lower in the vaccinated group. Weighing at the end of the trial took place at a comparable age (c=58 days, v = 62 days). Statistical analysis was done using SOFAstats version 1.4.5. P-values < 0.05 were considered significant.
Results: The average weight in the unvaccinated control group was 19.2kg at an age of 58 days. The average weight of the vaccinated group was 19.5kg at an age of 62 days. The average daily growth post weaning was 377g in the unvaccinated group and 382g in the vaccinated group. The standard deviation of the weights was 3.8kg in the control group and 3.4kg in the vaccinated group. The coefficient of variation was 20% in the control group and 17% in the vaccinated group. The weights were normally distributed in both groups. There was no statistically significant difference in weight between both groups (P=0.437, t-test). The variation in weights was significantly less in the vaccinated group (P=0.022, Mann Whitney U test).
Conclusion: The effect of reduction of losses due to vaccination with Ecoporc SHIGA® is well documented. In practice, there is the impression that vaccinated pigs also show a better growth and especially a better uniformity mostly because of a smaller number of pigs with growth retardation. These results show that there is no improvement in growth, but that uniformity is better in the vaccinated group than in unvaccinated group after vaccination with Ecoporc SHIGA®. Although both groups of piglets were housed in different compartments, these data present the first indication that vaccinating against ED with Ecoporc SHIGA® can also improve uniformity of the piglets.

Disclosure of Interest: None Declared

Keywords: E. coli STEC, Ecoporc shiga, performance improvement

Bacteriology and Bacterial Diseases
E.COLI

PO-PF3-058

PASSIVE PROTECTION IN PIGS AGAINST EDEMA DISEASE PROVIDED BY AN INNOVATIVE COLI-CLOSTRIDIAL VACCINE FOR SOWS

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Introduction: Vaccinating sows pre-farrowing against neonatal scours caused by infections with *Escherichia (E.) coli* and *Clostridium (Cl.) perfringens* to provide passive protection to the off-spring during the first days of life is a routine measure on many sow farms. To our knowledge any attempt to develop a sow vaccine that provides passive protection against edema disease after weaning caused by Shiga-toxin producing E. coli strains expressing F18ab fimbria has failed to date. The study presented here describes a challenge experiment evaluating passive protection against edema disease in weaned pigs provided by a newly developed commercial sow vaccine.
Materials and Methods: In total 64 piglets were included in the challenge study. Pigs were obtained from 8 non-vaccinated sows and 8 sows vaccinated intramuscularly with 2 ml of a commercial Coli-Clostridia vaccine (Entericolix©) at 7 and 3 weeks pre-farrowing. Four piglets were randomly selected from each mother. Piglets were weaned at 21 days and were orally challenged at 28 days of age (7 days after weaning) with a virulent Shiga-toxin producing *E. coli* strain expressing adhesin F18ab. After challenge all animals were daily observed for 8 days post infection for development of clinical signs related to oedema disease. Clinical scoring was done using a method previously described (MacLeod et al. 1991). Results of the two groups were statistically compared using two-sided Mann-Whitney U-test, subsequently confirmed by Kolmogorov-Smirnov test (Bonferroni's adjusted p-level of significance p<0.005) for evaluating the clinical scores and Fisher's exact test, for mortality and morbidity rates.
Results: All clinical parameters were statistically significant different between the two treatment groups with clinical scores, prevalence of severe diarrhea (morbidity) and mortality being lower in the pigs born to the vaccinated sows. The mean global clinical scores were 10.34±12.00 and 1.14±3.22 in naïve and treated piglets respectively. Morbidity and mortality percentages were 68.75% (22/32) and 25.00% (8/32) respectively in naïve piglets vs. 18.75% (6/32) and 3.125% (1/32) in passive immunized animals.
Conclusion: In this study, the vaccination of sows pre-farrowing provided passive protection against clinical disease and mortality in the off-spring after challenge with a virulent Shigatoxin-producing E. coli strain expressing F18ab fimbriae. To our knowledge this is the first report describing successful protection against edema disease via passive immunization in weaned pigs at 28 days of age. The long lasting protection may be attributed to the innovative water-in-oil-in-water adjuvant specifically developed for sow vaccines.

Disclosure of Interest: None Declared

Keywords: None

Bacteriology and Bacterial Diseases
E.COLI

PO-PF3-209

Field trial with Parofor® for the treatment of post-farrowing diarrhoea

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Introduction: This abstract describes the efficacy of Parofor® (100 mg paromomycin sulphate per gram), for the treatment of diarrhoea in piglets post-farrowing. Paromomycin is an aminoglycoside and well recognized and investigated for its antiprotozoal activity.
Materials and Methods: A 300 sow farm in France, suffered from recurrent diarrhoea in the farrowing house, mainly in litters from first and second parity sows. Culture only revealed hemolytic *E. coli*, sensitive to most antibiotics such amoxicillin / clavulanic acid, colistin, aminoglycosides and fluoroquinolones. PCR for PED and rotavirus (A&C) was negative. Four consecutive batches were followed to evaluate efficacy of two treatments (n=168). In batch 1 (41sows), all affected piglets originated from first and second litter sows (n=16) and were treated once a day, curatively with amoxicillin / colistin 0.5cc intramuscular (Potencil®) and rehydration salts in the drinking water, for 2-4 consecutive days (=Group A/C). In the second batch (41sows), also all affected piglets came from first and second litter sows (n=16) and received 1g of Parofor® per piglet per day for 3 consecutive days in the drinking water together with rehydration salts (=Group P-C). The third and fourth batches (86 sows) were treated metaphylactic (as from 2 days after birth) with Parofor® at the same dosing regimen, (Group P-M). Measured parameters were: number of live born and weaned piglets, percentage mortality, weight at birth and at weaning. Also the economic difference between the groups was calculated using the PIGSIM calculator from IFIP.
Results: Number of live born for the A/C group, the P-C group and the P-M groups were respectively 13.9 and 13.8 and 13.8. A difference in percentage mortality and consequently weaned piglets was remarked: 22.2% and 10.8 weaned piglets in the A/C group versus respectively 17.4% and 11.4 in the P-C group and 14.2% and 11.8 in the P-M group. The average litter weight at birth and at weaning in the A/C group were 20.6kg and 77.8kg. In the P-C group, they were 20.7kg and 83.2kg. In the P-M group they were 19.5kg and 90.3kg. An economic gain between the A/C and P-C group was calculated 3.4€ per sold pig, corresponding with 77€ per sow per year. The economic difference between the A/C and P-M group was calculated 5.6€ per sold pig or 132€ per sow per year.
Conclusion: Next to the susceptibility of the main causative agents, also the pharmacokinetic behavior might impact the clinical outcome. Oral application of Parofor® results in very high concentrations of paromomycin (> 5000µg/ml) in the intestinal tract. Supposedly, the ease of product distribution made piglet treatment even more effective and reduced the contamination between litters.

Disclosure of Interest: None Declared

Keywords: paromomycin diarrhoea piglet

Bacteriology and Bacterial Diseases
E.COLI

PO-PF3-142

Losses and amount of antimicrobial treatment due to oedema disease- Effect of vaccination with Ecoporc SHIGA evaluated on 179 German farms

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Introduction: Oedema disease (ED), caused by Shigatoxin 2e (Stx2e) producing *E.coli* (STEC), occurs worldwide. Due to its high mortality rate and the high number of runts after an outbreak, it causes severe economic losses and also lots of physiological distress to the farmers. To prevent and fight the disease often high amounts of antimicrobials are needed. In this study the reduction of mortality and the influence on the use of antimicrobials was followed in 179 farms all over Germany before and after vaccination with Ecoporc SHIGA was implemented.
Materials and Methods: Data on the overall mortality rate- and the use of antimicrobials during nursery was collected on farms with clinical signs of ED. In the second step, the detection of STEC via bacterial culture and subsequent isolate typing via PCR confirmed the diagnosis. During the nursery period, the overall mortality rate (not only due to ED), the number of runts and the amount of Colistine used to prevent disease were investigated. The data of the study are based on the comparison of the performance of the piglets in the nurseries 6 months before and 6 months after implementing the vaccination with Ecoporc SHIGA. The average results were analyzed with the Wilcoxon test and p-values ≤ 0.05 were considered significant.
Results: The average number of total losses in the 179 farms during the 6 months before vaccination was 8.5% between weaning (at an average weight of 7.4kg) and end of nursery (at an average weight of 28,3kg). This was reduced to an average of 2.2% losses in the 6 months after vaccination started. Therefore, an improvement of 6.4% in the mean could be shown six months after vaccination ($p < 0.001$). Sixty farms used Colistine to reduce ED before they started vaccinating. Thirty-two of them were able to stop using Colistine completely; the rest were able reduce the length of treatment, but would not stop the use completely due to diarrhoea in the weaners. This reduced the average use of Colistine of 4.0 days before vaccination to 1.6 days after vaccination ($p < 0.001$).
Conclusion: The data of 179 German farms show that Ecoporc SHIGA can significantly reduce the overall mortality rate during the nursery phase. Furthermore, the reduction of the average number of days of use of Colistine (4.0 to 1.6 days) shows that Ecoporc SHIGA can also meaningfully contribute to the reduction of the use of antimicrobials in pigs, which is one of the key goals of the EU for a sustainable production of livestock.

Disclosure of Interest: None Declared

Keywords: Oedema disease, Shigatoxin, Vaccination

Poster Abstracts

Bacteriology and Bacterial Diseases
E.COLI

PO-PF3-281

Reduction of antibiotic use to treat post-weaning diarrhoea following oral vaccination with non-pathogenic Escherichia coli F4: case report

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Introduction: Post-weaning Escherichia coli diarrhea (PWD), also called post-weaning enteric colibacillosis, in pigs remains a major cause of economic losses for the pig industry, due to either piglet death, or poor weight gain in surviving piglets. PWD typically causes mild to severe watery diarrhea between 5 and 10 days after weaning and is caused primarily by enterotoxigenic Escherichia coli (ETEC). The most common adhesins found on ETEC from PWD in pigs are associated with fimbriae F4 (previously called K88) and F18, while the predominant enterotoxins are LT, STa and STb. The presented case report shows the impact of vaccination with a live non-pathogenic E. coli F4 vaccine on overall post-weaning piglet health.
Materials and Methods: For more than 2 years, a closed 400-sow farm in a 3-week batch management system suffered from severe problems with post-weaning diarrhea. The clinical phase started already within 3 days post-weaning with high levels of mortality and massive use of in-feed and injectable antibiotics. During that period, several preventive options have been applied without success. Treatment incidence (treatments per 100 piglets), using enrofloxacin injections, before vaccination was 300. Piglets were weaned at 24 to 26 days of age. Following diagnosis of an ETEC F4, cause of PWD, an oral vaccination with a live non-pathogenic strain of E. coli (Coliprotec®F4; Prevtec Microbia) 7 days before the clinical outbreak was performed at a dose of 2 ml per piglet according to manufacturers' instructions. The following parameters were recorded for each group: number of dead piglets (% mortality) d1-d10, number of antibiotic injections, treatment incidence (TI), % pens treated on total, day of change to post-weaning diet.
Results: After vaccination with Coliprotec®F4, piglet mortality in the first 10 days decreased from 4.7% to 1.0%. A significant decrease ($P < 0.05$) in TI was observed from 300 to 30 after vaccination. Feeding change post-weaning could be performed at least 3 days earlier, indicating a higher feed intake throughout the early post-weaning period.
Conclusion: This case report shows that following a proper diagnosis confirming the presence of ETEC F4 in post-weaning diarrhea problems, vaccination with an oral live non-pathogenic E. coli F4 vaccine (Coliprotec®F4) shows improvement of several economically important parameters. Vaccination with Coliprotec®F4 resulted in lower antibiotic use (90% reduction in TI), no zinc oxide inclusion in the feed and decreased piglet mortality.

Disclosure of Interest: None Declared

Keywords: antibiotic reduction, ETEC F4, vaccination

Bacteriology and Bacterial Diseases
E.COLI

PO-PF3-293

COMPARISON OF ANTIMICROBIAL RESISTANCE OF ESCHERICHIA (E.) COLI ISOLATED FROM NURSERY PIGLETS WITH DIARRHEA AND FROM THEIR DRINKING WATER

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Introduction: A common problem in swine industry is diarrhea in nursery pigs, causing losses by weight loss, mortality, dehydration and medication. Enterotoxigenic *E. coli* (ETEC) is the main agent of post-weaning diarrhea. Transmission is by fecal-oral route, and water may be an important contamination source. The aim of the study was to compare the antimicrobial (AMB) resistance of *E. coli* and ETEC isolated from pigs with post-weaning diarrhea to *E. coli* isolated from water of the same nurseries, by antibiogram technique.
Materials and Methods: 15 rectal swabs from piglets with post-weaning diarrhea and one water sample were collected from each of 10 nurseries in southern Brazil. After bacteriological isolation, 4 water samples isolated *E. coli* and from the 60 rectal swab samples, 21 isolated *E. coli.* Thus, those 25 isolates were submitted to virulence factors multiplex PCR analysis, identifying ETEC (7 rectal swab) and non-ETEC (4 waters, 14 rectal swab) specimens. The 25 *E. coli* isolates were submitted to disk diffusion antibiogram as described by CLSI protocols. The AMB agents (Oxoid®) tested were: Apramycin (15µg), Colistin (25µg), Florfenicol (30µg), Fosfomycin (50µg), Gentamicin (10µg), Lincomycin (10µg), Lincomycin+Espectinomicin (10µg), Neomycin (30µg), Oxitetracycline (30µg) e Sulfametoxazol+Trimetoprim (25µg).
Results: The 7 ETEC rectal swab's isolates were resistant to lincom, and just one was resistant to fosfom (14.2%). All 14 *E. coli* from rectal swabs were resistent to florfen, and none of the samples presented resistance to colist. Amongst the 4 *E. coli* isolates from water, 75% were resistant to at least one of the AMBs apram, florfen, oxitet, and sulfa+trimet; there was no resistance to colist and fosfom. All isolates presented resistance from at least one, to 9 AMBs. 24 (96%) of the *E. coli* isolates presented resistance to at least 3 different groups of AMBs.
Conclusion: *E. coli* isolates showed high resistance to florfen and neom, but ETEC isolates did not. All the isolates presented high resistance to 3 AMBs: lincom, lincom+espectin and sulfa+trimet, showing that similar AMB resistance profiles can exist among specimens with different pathogenicity. 5 ETEC with the same virulence factors (F18, Stap and Stb) were resistant to lincom and sulfam+trimet. From those 5 ETECs, at least two were resistant to apram, colist, florfen, gentam, and lincom+spectin, showing a possible relation between virulence factors and AMB resistance. The 3 groups of bacteria presented resistance to at least 3 AMBs from different groups, representing multi-drug resistant bacteria. ETEC showed low resistance to fosfom, allowing a prudent AMB choice to swine industry.

Disclosure of Interest: None Declared

Keywords: ANTIBIOGRAM, MULTI-DRUG RESISTANCE, POST-WEANING DIARRHEA

Bacteriology and Bacterial Diseases
E.COLI

PO-PF3-145

Prevalence of virulence factors of Escherichia coli isolated from piglets with post-weaning diarrhoea in Belgium and The Netherlands.

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Introduction: Post-weaning Escherichia coli diarrhea (PWD), also called post-weaning enteric colibacillosis, remains a major cause of economic losses for the pig industry, due to either piglet death, or poor weight gain in surviving piglets. PWD typically causes mild to severe watery diarrhea between 5 and 10 days after weaning and is caused primarily by entero-toxigenic Escherichia coli (ETEC). The most common adhesins found in ETEC from pigs with PWD are fimbriae F4 (previously called K88) and F18, while the predominant enterotoxins are LT, STa and STb. The objective of the present study is to determine the prevalence ETEC subtypes causing PWD in Belgium and The Netherlands.
Materials and Methods: A total of 305 pig herds distributed in the Benelux (Belgium, n=144 and The Netherlands, n=161) showing clinical signs of PWD (sudden death, watery diarrhea, decreased feed consumption, dehydration, depression) were sampled between January 2014 and December 2015. Rectal swab samples from diarrheic pigs obtained within 48 hours of the start of the outbreak and/or ileum swab samples at necropsy from 5 piglets per farm were collected and submitted to DGZ-Vlaanderen (Torhout, Belgium) for diagnostic investigations. The presence of virulence factors - adhesins (F4, F5, F6, F18 and F41) and toxins (LT, STa, STb, Stx) - was analysed by PCR at IZSLER (Brescia, Italy).
Results: In total, 215 non-hemolytic and 487 hemolytic E. coli strains were isolated and subsequently tested by PCR. The prevalence of the different ETEC subtypes was as follows: F4-ETEC (27.8%) and F18-ETEC (19.1%). Other subtypes such as F5-ETEC (0.7%) and F6-ETEC (0.6%) were occasionally detected. On a herd level, the prevalence of the different ETEC subtypes was as follows: F4-ETEC (46.5%) and F18-ETEC (35.9%).Besides ETEC, 20 isolates (2.8%) were classified as Shiga toxin-producing E. coli (STEC). The most prevalent virotypes in the necropsy study in Belgium were F18, STa, STb (12.4%); F4, STa, STb (10.6%); F4, STb, LT (6.9%) and F4, STa, STb, LT (5.0%). In The Netherlands, the virotypes more frequently detected were F18, STa, STb (9.9%); F4, STa, STb, LT (9.3%); F4, LT (5.9%) and F4, STa, STb (3.7%).
Conclusion: This study confirms that the fimbriae type F4 is more prevalent than F18 among E. coli isolates from PWD cases in Belgium and The Netherlands. ETEC strains were involved in nearly 47.2% of the cases investigated. Laboratory diagnostics, including characterization of virulence factors, are essential to understand the role of E. coli in PWD outbreaks and initiate appropriate preventive and control measures such as oral vaccination.

Disclosure of Interest: None Declared

Keywords: ETEC, prevalence, virulence factors

Bacteriology and Bacterial Diseases
E.COLI

PO-PF3-098

Diagnostic options for Escherichia coli associated with post-weaning diarrhoea: when to use what test?

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Introduction: Post-weaning Escherichia coli diarrhea (PWD), also called post-weaning enteric colibacillosis, in pigs remains a major cause of economic losses for the pig industry, due to either piglet death, or poor weight gain in surviving piglets. PWD typically causes mild to severe watery diarrhea between 5 and 10 days after weaning and is caused primarily by enterotoxigenic Escherichia coli (ETEC). The most common adhesins found on ETEC from PWD in pigs are associated with fimbriae F4 (previously called K88) and F18. In order to obtain a rapid diagnosis, a new on-site test kit for field use has become available that can detect the most important adhesion factors (F4, F18) involved in PWD cases. The objective of the present study is to determine the sensitivity (Se), specificity (Sp) and the potential use for this new tool in diagnosing F4 and F18 E. coli.
Materials and Methods: A total of 40 pig herds distributed in the Benelux showing clinical signs of PWD were targeted. Fecal samples were collected from diarrheic pigs of a total of 5 piglets per farm and submitted to DGZ-Vlaanderen (Torhout, Belgium). Besides the PCR test for the presence of virulence factors (F4, F18) (ISZLER, Emilia Romagna) on bacterial isolates of hemolytic and non-hemolytic E. coli, another test was performed on all fecal samples in order to compare the diagnostic results: lateral immunochromatography (LIC) test (Rainbow Piglet F4/F18; Bio-X Diagnostics, Rochefort, Belgium). Results expressed as positive or negative for F4 and F18 for both tests (LIC, PCR) were pairwise compared with PCR results as the 'golden standard' in order to calculate both sensitivity and specificity of the rapid diagnostic tools.
Results: In total, 183 samples were collected according to the rules of the sampling protocol. The test characteristics of the lateral immunochromatography as compared to PCR as the 'golden standard' were as following for F4: Se 50%, Sp 87% and for F18: Se 57%, Sp 91%.
Conclusion: The results from the present study show that in case of negative results, the probability of having no adhesion factors expressed is quite high (Sp > 87% overall for F4 and F18). Taking into account the lower values obtained for the sensitivity, we have to keep in mind that not all positive results are detected as such. In addition, the presence of E. coli strains with F4 or F18 fimbria but without enterotoxins is not uncommon. Therefore, in order to confirm the presence of ETEC, the use of bacteriology and subsequent PCR test, with detection of fimbriae and toxins, is crucial.

Disclosure of Interest: None Declared

Keywords: diagnostics, Escherichia coli, on-farm test

Poster Abstracts

Bacteriology and Bacterial Diseases
E.COLI

PO-PF3-250

Increased weight gain in a farm with chronic post-weaning diarrhea following oral vaccination with non-pathogenic Escherichia coli F4: case report

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Introduction: Post-weaning Escherichia coli diarrhea (PWD), also called post-weaning (pw) enteric colibacillosis, in pigs remains a major cause of economic losses for the pig industry, due to either piglet death, or poor weight gain in surviving piglets. PWD typically causes mild to severe watery diarrhea between 5 and 10 days after weaning and is caused primarily by enterotoxigenic Escherichia coli (ETEC). The most common adhesins found on ETEC from PWD in pigs are associated with fimbriae F4 (previously called K88) and F18, while the predominant enterotoxins are LT, STa and STb. The presented case report shows the impact of oral vaccination with a live non-pathogenic E. coli F4 vaccine on overall pw piglet health.
Materials and Methods: For more than 2 years, a closed 600-sow farm suffered from severe problems with PWD. The clinical phase started within 5 days pw with moderate levels of mortality, increased diarrhea scores and large variation in final piglet weight. During that period, several preventive options have been applied without success. Piglets were weaned at24 to 26 days of age. Following diagnosis of an ETEC F4, cause of PWD, an oral vaccination with a live non-pathogenic F4-positive strain of E. coli (Coliprotec®F4; Prevtec Microbia) 7 days before the clinical outbreak was performed at a dose of 2 ml per piglet according to manufacturers' instructions. A total of 3 week groups were enrolled, of which half of the litters were vaccinated (non-medicated) and compared to non-vaccinated and non-medicated litters. The following parameters were recorded for each group: number of dead piglets (% mortality), number of sold piglets, weaning weight, selling weight, days in pw section, total feed consumption and feed conversion.
Results: Mortality did not differ between both treatment groups and was at 3.12%. The incidence of severe diarrhea was decreased and piglet selling weight was higher ($P > 0.05$) in the vaccinated group (19.58 ± 0.28 kg) than in the control group (18.35 ± 0.63 kg). Average daily weight gain over a 41.67-day period in the pw section was slightly better in the vaccinated group (334 ± 0.011 g) than in the control group (304 ± 0.014 g). Taking into account all costs of production (incl. vaccine cost) during the pw phase, the economic improvement of Coliprotec®F4 vaccination was 42 cents per produced piglet.
Conclusion: This case report shows that following a proper diagnosis confirming the presence of ETEC F4 in PWD problems, vaccination with an oral live non-pathogenic E. coli F4 vaccine (Coliprotec®F4) shows improvement of piglet daily weight gain, overall piglet selling weight and profitability in a chronically PWD-affected farm.

Disclosure of Interest: None Declared

Keywords: ETEC F4, increased weight gain, vaccination

Bacteriology and Bacterial Diseases
E.COLI

PO-PF3-164

Prevalence of virulence factors in Escherichia coli isolated from pigs with post-weaning diarrhoea in Spain.

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Introduction: Post-weaning diarrhoea (PWD) is a major cause of economic losses to the pig industry from both mortality and reduced growth rates (*Fairbrother et al., 2005*). PWD is mainly caused by some strains of *E. coli*, known as enterotoxigenic *E. coli* (ETEC), with the ability to produce one or several enterotoxins, including heat-labile toxin (LT), heat-stable toxin a (STa) and heat-stable toxin b (STb), and attach to intestinal cells. The fimbrial adhesins most commonly found in ETEC causing PWD in pigs are F4 (formerly K88) and F18. In spite of its importance, recent data on the prevalence of *E. coli* virulence factors causing PWD in Spain is limited.
The objective of the present study is to investigate the prevalence of ETEC virulence factors involved in cases of PWD in Spain.

Materials and Methods: A total of 348 samples from Spanish farms (n=106) with a recent history of PWD in nursery pigs (sudden death, watery diarrhoea, decreased feed consumption, dehydration and depression) were included in the study during 2015. Rectal swabs from pigs with diarrhoea were collected within 48 hours of the start of the outbreak and submitted to the laboratory (Labocor; Madrid, Spain) for *E. coli* diagnostic investigation. DNA from a single *E. coli* colony per sample was extracted and the presence of adhesins (F4, F5, F6, F18 and F41) and toxins (LT, STa and STb) tested by PCR at IZSLER (Brescia, Italy).
Results: *E. coli* isolates were recovered from all of the samples submitted and virulence factors identified in 197 of them (56.6 %). Genes for STb, STa, and LT were detected in 40.7%, 26.8% and 36.9% of the isolates. Regarding fimbrial adhesin genes, F4 was detected in 21.5% of the samples and F18 in 25.9 of them. No F5, F6 or F41 genes were detected.
One hundred and fifty nine isolates (45.7%) were classified as ETEC based on their combination of fimbriae and toxins. From these, 56.6% belonged to the subtype F18-ETEC and 43.4% were F4-ETEC. Six isolates were positive by PCR for the fimbrial adhesin gene encoding for F4 but were negative for toxins.
F4-ETEC subtype was identified as the cause of PWD in 20.8% of the farms, whereas F18-ETEC was found as single subtype in 34.0% of the cases. In 8.5% of the farms, both F4-ETEC and F18-ETEC were identified.

Conclusion: This study shows that ETEC is a prevalent cause of PWD in nursery pigs in Spanish pig farms. F4-ETEC and F18-ETEC are important subtypes involved in the aetiology of this disease. Laboratory diagnostics, including characterization of virulence factors, are essential to understand the role of different *E. coli* isolates in PWD outbreaks and initiate appropriate preventive and control measures.

Disclosure of Interest: P. J. Sánchez Conflict with: Elanco Animal Health, A. Hidalgo Conflict with: Elanco Animal Health, P. Núñez Conflict with: Elanco Animal Health, L. Pérez Conflict with: Elanco Animal Health

Keywords: ETEC, post weaning diarrhoea, prevalence

Bacteriology and Bacterial Diseases
E.COLI

PO-PF3-121

Virotyping of E. coli isolated from swine from 2013 until mid 2015

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Introduction: *E. coli* occurs in a multitude of variants. In addition to commensal, naturally occurring strains found in the intestinal flora of all mammals, there are also pathogenic strains classified as intestinal (InPEC) or extra-intestinal *E. coli* (ExPEC). Identification of these genes that code for virulence factors makes it possible to determine the potential virulence of *E. coli* isolates and to distinguish between different pathotypes.
Materials and Methods: A total of 2803 *E. coli* were isolated from swine during diagnostic examinations and analyzed for virulence factors by multiplex PCR. While the *E. coli* isolated in 2013 (n: 849) were analyzed only for the genes of fimbriae F4, F5, F6, F18, and F41, those isolated in 2014 (n: 1138) and in the first half of 2015 (n: 816) were analyzed for 16 further genes coding for fimbriae, other adhesins, and toxins. Results were used to determine the *E. coli* pathotype, and the distribution and combination of factors were analyzed. Where information about the pigs' age was available, the distribution of factors was also analyzed for age groups.
Results: In all *E. coli* isolated (n: 1231) from suckling piglets, 14.7% (n: 74) showed the gene for F4 fimbriae, while that for F18 was found in only 4.2% (n: 21). In weaning piglets, the gene for F4 fimbriae was detected in only 9.0% (n: 502) of all *E. coli* isolated, and that for F18 in 19.9% (n: 100).
As expected, the most frequently detected fimbrial genes of *E. coli* (n: 1954) coded for *fimH* (81.6%) and *fimA* (73.6%), mostly in combination (total n: 1954); the others were *paa* (10.2%), *aidA* and *papC* (9.8% each), *intimin* (7.3%), *F18* (6.7%), and *F4* (6.2%).
In 1954 *E. coli* analyzed, 85 (4.4%) showed the *stx2e* gene, coding for the Shiga toxin variant 2e, which is characteristic of STEC, including EDEC. Of those *E. coli* with the *stx2e* gene, 58% (n: 49) additionally showed the gene for F18 fimbriae, which are characteristic of the classical agent for edema disease in swine. The pathotype was determined for 479 (24.5%) of 1954 *E. coli* isolates according to strong classification criteria. Of these pathotypes, 54.5% showed hemolysis, whereas 45.6% did not. The dominant pathotype was ETEC (56.7%, n: 276), followed by EPEC (28.5%, n: 136) and EDEC (10.3 %, n: 29).
Conclusion: Virotyping of *E coli* isolates was found to be a helpful tool which made it possible to determine the potential virulence of strains and their etiological relationship with swine disease in almost 25% of cases. Furthermore, the analyses showed that there may be more potentially virulent *E. coli* strains, whose combination of virulence factors does not fit in the known classification scheme.

Disclosure of Interest: None Declared

Keywords: pathotype, virulence

Bacteriology and Bacterial Diseases
E.COLI

PO-PF3-301

CLINICAL EFFECT OF APRAVET® 100 G/KG PREMIX IN PIGS NATURALLY INFECTED WITH ESCHERICHIA COLI

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Introduction: As well documented in scientific literature, apramycin (APRM) has been evaluated for the treatment of intestinal bacterial infections in animals. Despite poor absorption from the gastrointestinal tract, oral administration of apramycin has resulted in clinical efficacy in the treatment of colibacillosis in pigs. The objective of this study was to assess the clinical effect of Apravet® 100 g/kg premix used in pigs naturally infected with *Escherichia coli*.

Materials and Methods: Six groups of 4 pigs (Danube white), of both sexes (8.8-10.5 kg), 4-5 weeks of age, were used. All pigs were naturally infected with *E. coli*. On day 0 of the trial, the infected groups (II-VI) were treated via feed with 8 mg apramycin (APRM) per kg bodyweight (BW) as Apravet® 100 g/kg premix for 21 consecutive days. efficacy of the medication was evaluated by observing the clinical symptoms, scoring system (faecal consistency: 0=normal, 1=soft consistency, 2=mild diarrhoea, 3=severe diarrhoea and faecal colour: 1=yellow, 2=green, 3=brown) and results of bacteriological examinations (after euthanasia and necropsy of all pigs) of *E. coli* from the gastrointestinal tract. the results of the examination were determined according to t-test of Student-Fisher.
Results: During the study, specific clinical symptoms of colibacillosis and changes in consistency and color of faeces were observed in the infected unmedicated group (I). All animals in the control group showed clinical signs and had a faecal consistency score of 1.48. None of the animals in the treated group showed clinical signs and the mean faecal score was 0.33, significantly lower in comparison with the control group. Included in the feed of infected pigs (II-VI) at a dose of 8 mg APRM per kg BW, Apravet® leads to statistically significant decrease in the pathological parameters
Conclusion: The results from the study show that Apravet® administration via feed at 8 mg per kg BW has significant therapeutic effects in pigs naturally infected with *E. coli*. APRM concentrates in the small and large intestines which may have a significant role in clinical efficacy against bacterial enteritis in pigs, especially to treat and overcome gastrointestinal symptoms caused by *E. coli*.

Disclosure of Interest: None Declared

Keywords: Ecoli apramycin efficacy

Poster Abstracts

Bacteriology and Bacterial Diseases
E.COLI

PO-PC02-007

Field efficacy of Coliprotec® F4, live oral vaccine against post-weaning diarrhoea caused by F4-enterotoxigenic E. coli (F4-ETEC), in German pig farms

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Introduction: Nowadays, post-weaning diarrhoea (PWD) remains a major cause of economic losses for the pig industry. PWD is mainly caused by enterotoxigenic *Escherichia coli* (ETEC), with F4-ETEC being highly prevalent in Europe. Over the years, a number of preventive measures have been recommended to minimize the impact of PWD, including the use of antimicrobials. However, an increase in antimicrobial resistance among *E. coli* recovered from PWD cases has been recently reported. Coliprotec® F4 is a live non-pathogenic *E. coli* vaccine registered in Europe for active immunization of pigs against PWD caused by F4-ETEC that can be administered from 18 days of age as drench application or in drinking water. This study investigates the efficacy of Coliprotec® F4 against PWD caused by F4-ETEC in German pig farms.
Materials and Methods: Two separate studies were conducted in German pig farms with a history of PWD caused by F4-ETEC following GCP standards. Piglets of at least 18 days of age were randomly allocated to vaccinated or control (non-vaccinated) groups. Coliprotec® F4 (Prevtec Microbia) was administered to piglets (vaccinated group) at weaning (at least 18 days of age) via drench application (*Study A*, n=343) or in drinking water via bowls (*Study B*, n=351). Tap water was administered instead to non-vaccinated piglets at the same time (*Study A*, n=354; *Study B*, n=358). Diarrhoea was investigated at individual pig level daily for 21 days post vaccination [Faecal score: 0 (normal), solid faeces; 1 (slight), soft faeces; 2 (mild), presence of liquid but more solid particles than liquid; 3 (moderate), more liquid than solid particles; 4 (severe), liquid, watery diarrhoea].
Results: Piglets vaccinated with Coliprotec® F4 showed a significant reduction of moderate to severe diarrhoea in the 21-day post vaccination period, independently of the mode of administration. In *Study A*, 3.8% of pigs vaccinated by drenching presented moderate to severe diarrhoea compared to 22.3% of pigs in the control group ($p<0.001$). *Study B* showed a significant reduction of moderate to severe diarrhoea in piglets vaccinated via bowls compared to the control group (4.9% and 11.5%, respectively; $p=0.001$). In both farms, F4-ETEC was confirmed as the cause of PWD in the batch of pigs preceding the start of the study by PCR. No vaccine related adverse events were observed during the studies.
Conclusion: Coliprotec® F4 administered via drench application or drinking water reduced significantly the incidence of moderate to severe diarrhoea in pigs with PWD caused by F4-ETEC in commercial pig farms. Vaccination of piglets with Coliprotec® F4 should be considered as part of preventive programs for PWD.

Disclosure of Interest: None Declared

Keywords: F4-ETEC, Post-weaning diarrhoea, Vaccine

Bacteriology and Bacterial Diseases
E.COLI

PO-PF3-266

Vaccination of suckling piglets with Coliprotec® F4 in a French farm dealing with post-weaning diarrhea: A case report

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Introduction: Post-weaning diarrhea (PWD) remains a major cause of economic losses for the pig industry. PWD is mostly caused by enterotoxigenic *Escherichia coli* (ETEC), characterized by the production of fimbriae (F4 and F18) and enterotoxins (LT, STa and STb). A number of measures have been recommended to control and minimize the impact of PWD. Moreover, an increase in antimicrobial resistance among *E. coli* recovered from PWD cases has been recently reported. Coliprotec F4 is a live non-pathogenic *E. coli* vaccine registered in Europe for active immunization against PWD caused by F4-ETEC. This case report describes the vaccination of suckling piglets with Coliprotec F4 in a French farm to control PWD.
Materials and Methods: This study reports on a 140 sow farrow-to-finish farm located in France with a 7-batch farrowing system and a history of PWD. After weaning (28 days of age), complete batches of piglets are transferred to a first-stage nursery for two and half weeks, and then, to a second-stage nursery for five weeks, both managed in all-in, all-out flows. Dry feed specifically formulated to address PWD but without antibiotics is used during this period. Before implementing Coliprotec F4 vaccination, water medication for controlling PWD was used consisting of oxolinic acid (20 mg/kg/day for 5 days) and/or neomycin (20 mg/kg/day for 5 days). In spite of those treatments, PWD still occurred 4 to 5 days after weaning. Samples of clinical cases were taken and laboratory diagnostic confirmed that F4-ETEC was the causative agent of PWD in this farm. In order to optimize the control of PWD, vaccination with Coliprotec F4 of suckling piglets at 21 days of age (7 days pre-weaning) was initiated. Number of dead pig and body weight, average daily gain (ADG) and antibiotic treatments were recorded for two batches before (456 piglets) and after (432 piglets) the implementation of Coliprotec F4 vaccination.
Results: Following vaccination of piglets with Coliprotec F4, PWD clinical signs decreased and specific antibiotic treatments for PWD was not required in any of the two vaccinated batches. Overall, mortality decreased in the nursery from 5.04% to 1.39% ($p<0.05$). Considering only the first stage, mortality dropped from 2.85% to 1.39% ($p=0.07$) and from 2.26% to 0.0% during the second stage ($p<0.05$). Whereas the ADG in this nursery was already high before starting the vaccination, ADG remained above the national average after Coliprotec F4 vaccination.
Conclusion: Vaccination of piglets one week before weaning with Coliprotec F4 reduced PWD clinical signs, mortality and antibiotic treatments during the nursery in this farm with a history of PWD caused by F4-ETEC.

Disclosure of Interest: None Declared

Keywords: post weaning diarrhoea, vaccine

Bacteriology and Bacterial Diseases
E.COLI

PO-PC02-009

ANTIMICROBIAL RESISTANCE OF ENTEROTOXIGENIC ESCHERICHIA COLI (ETEC) ISOLATED FROM POST-WEANING DIARRHOEA OUTBREAKS IN ITALY

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Introduction: Post-weaning diarrhea (PWD), due mainly to Enterotoxigenic *Escherichia coli* (ETEC), is an economically important disease for the swine industry. Over the years, a number of measures have been recommended to address risk factors and minimize the impact of PWD, including feeding or management strategies. Moreover, antimicrobials have been frequently used for treating and controlling PWD. The aim of this study is to investigate the antimicrobial resistance of ETEC isolates from recent cases of PWD in Italian pig herds.
Materials and Methods: A total of 138 samples (rectal swab, intestine or entire piglet) obtained from 46 farms with PWD located in Northern Italy were submitted to IZSLER (Italy) for diagnostic investigation between June 2014 and November 2015. Samples were obtained in the first 48 hours of the PWD outbreak, transported to the laboratory and cultured using standardized bacteriological methods. *E.coli* isolates were classified into pathotypes following PCR investigation of virulence factors. The susceptibility of ETEC isolates against 11 antimicrobial agents (apramycin, aminosidine, florfenicol, cefquinome, enrofloxacin, flumequine, thiamphenicol, gentamicin, tetracycline, trimethoprim-sulphamethoxazole and erythromycin) was determined using a disc diffusion method (Kirby-Bauer). CLSI and CA-SFM guidelines and interpretative criteria were followed and isolates were classified as resistant, susceptible or intermediate.

Results: In total, 46 *E.coli* isolates were obtained and 40 of them were classified as ETEC following identification of virulence factors by PCR (F4-ETEC, 55%; F18-ETEC, 42%). The percentage of ETEC isolates with decreased susceptibility (intermediate and resistant isolates) to the antimicrobials tested was: erythromycin (98%), tetracycline (90%), thiamphenicol (88%),trimethoprim-sulphamethoxazole (80%), apramycin (75%), florfenicol (65%), aminosidine (63%), flumequine (55%), gentamicin (55%), enrofloxacin (45%) and cefquinome (17%). All the isolates investigated showing decreased susceptibility to multiple antimicrobials and at least to two of them. In addition, 83% and 28% of the isolates were resistant to more than 6 and 9 antimicrobial agents, respectively.
Conclusion: In this study, decreased susceptibility among recent ETEC isolates to some of the antimicrobial agents commonly used against PWD in Italy is described. The development of antimicrobial resistance among ETEC isolates may compromise the treatment and control of PWD, representing an important problem and making necessary the implementation of alternative strategies for controlling PWD.

Disclosure of Interest: None Declared

Keywords: antimicrobial resistance, ETEC

Bacteriology and Bacterial Diseases
E.COLI

PO-PF3-265

Vaccination with Coliprotec® F4 at weaning in a French farm dealing with post-weaning diarrhea due to F4-ETEC: A case report

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Introduction: Post-weaning diarrhea (PWD) remains a major cause of economic losses for the pig industry. PWD is mostly caused by enterotoxigenic *Escherichia coli* (ETEC), a pathotype characterized by the production of fimbriae (F4 and F18) and enterotoxins (LT, STa and STb). A number of preventive measures have been recommended to control and minimize the impact of PWD. Moreover, an increase in antimicrobial resistance among *E. coli* recovered from PWD cases has been recently reported. Coliprotec F4 is a live non-pathogenic *E. coli* vaccine registered in Europe for active immunization of pigs against F4-ETEC. This case report describes the implementation of a vaccination program with Coliprotec F4 at weaning to control PWD in a French farm.
Materials and Methods: This study reports on a 250-sow farrow-to-finish farm with a 7 batches farrowing system and a history of PWD. After weaning at 28 days of age, complete batches of piglets are transferred to a nursery for two and a half weeks, managed in all-in, all-out flows. Then, piglets are moved to a finishing unit. A specific diet without antibiotic was formulated to address PWD during the nursery. Before implementation of Coliprotec F4 vaccination, water medication for controlling PWD was used in the nursery, with apramycin (10 mg/kg/day for 5 days) and/or neomycin (20 mg/kg/day for 5 days). In spite of those treatments, PWD still occurred 10 days after each weaning. F4-ETEC was confirmed as the causative agent of PWD in this farm. In order to optimize the control of PWD, it was decided to vaccinate piglets with Coliprotec F4 at 28 days of age (weaning day). Vaccination was initially performed via individual drenching and in subsequent batches in drinking water with a bowl at arrival in the nursery for labor and time optimization. Mortality, average daily gain (ADG) and antibiotic treatments were recorded for 3 batches before (915 piglets) and 7 batches after (2464 piglets) the implementation of Coliprotec F4 vaccination.
Results: Following vaccination of piglets with Coliprotec F4, PWD clinical signs decreased and specific antibiotic treatments for PWD were not required in any of the seven vaccinated batches. Mortality was decreased from 3.37% to 0.58% ($p<0.05$) during the nursery. ADG in the nursery was stable before and after Coliprotec F4 vaccination, with 254 g/day and 260 g/day, respectively ($p>0.05$).
Conclusion: In this farm, management of PWD was optimized by the implementation of Coliprotec F4 vaccination at weaning. That intervention improved clinical signs of PWD and mortality in the nursery and allowed a reduction in antibiotic treatments against PWD.

Disclosure of Interest: None Declared

Keywords: Post weaning diarrhoea, Vaccine

Poster Abstracts

Bacteriology and Bacterial Diseases
H.PARASUIS

PO-PF3-154

Molecular serotyping of Haemophilus parasuis from Taiwanese pigs

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Introduction: *Haemophilus parasuis* (Hps), the causative agent of Glässer's disease, is an important porcine pathogen leading to huge economic impact in swine industry worldwide. Fifteen serovars have been described and demonstrated differences in their virulence from highly virulent to non-virulent, but many strains are nontypeable with the current sera. Due to complete genome analysis, all Hps strains include non-typeable (NT) strains can be serotyping by molecular serotyping assay which was able to differentiate fourteen of the fifteen serovars of Hps except serovar 5 and 12. The molecular serotyping assay was an effective, fast, simple and useful epidemiological tool. However, there was no data shown molecular serotyping of Hps from Taiwanese pigs. The aim of this study was to serotype Hps from Taiwanese pigs by molecular serotyping assay.
Materials and Methods: Forty-four isolates were collected from pigs from middle and southern Taiwan from 2013 to 2015. If pigs observed gross lesions of polyserositis at necropsy, swabs sampled from meninges, pleura, pericardium, peritoneum and synovial cavity was sampled for Hps culture of performed on Chocolate agar at 37°C, 5% CO_2, 24 to 72 hours. Isolates were serotyping by molecular serotyping multiplex polymerase chain reaction (mPCR). PCR products were analyzed by 2% agarose gel at 120V for 40 minutes.
Results: Among the serotypes of the 44 isolates, 11 (25%), 6 (13.6%), 2 (4.5%), 2 (4.5%), 1 (2.3%), 1 (2.3%) and 1 (2.3%) belonged to serovars 5 or 12, 6, 4, 7, 2, 8, and 9, respectively. However, there were still 20 isolates (45.5%) belonged to NT strains because of appearance or disappearance of unexpected amplicons. Electrophoresis results of 4 isolates just only appeared species-specific (Sp-sp) marker. Eight isolates appeared Sp-sp marker with approximate 305, 830 and 1000 bp weak unexpected amplicons. Five isolates appeared Sp-sp marker with approximate 330 and 375 bp unexpected amplicons. Three isolates appeared Sp-sp marker with approximate 350 and 400 bp unexpected amplicons.
Conclusion: The results of this study indicated that perhaps there were some gene differences of Hps from sequenced strains or new serotypes from Taiwan. In the future, the correlation of serotype and genotype will be investigated.

Disclosure of Interest: None Declared

Keywords: Glässer's disease, Haemophilus parasuis, Molecular serotyping

Bacteriology and Bacterial Diseases
H.PARASUIS

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The determination of minimum inhibitory concentrations of selected antimicrobials for porcine Haemophilus parasuis isolates from the Czech Republic

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Introduction: Glasser's disease caused by *H.parasuis* (Hps) is present in all major swine- raising countries and Hps is considered as important pathogen in all production systems including age-segregated farm cooperations. Hps is heterogeneous bacteria with serotype variability which is causing difficulties for control by vaccination. Antimicrobial treatment is recommended to control severe outbreaks of systemic infection. The aim of this study is to evaluate antimicrobial susceptibility profiles for Hps isolates for the most important groups of antibiotics commonly used in field.
Materials and Methods: 30 isolates of Hps were obtained from diseased pigs from herds in the Czech Republic. The isolates were identified by a PCR and serotypization (coagglutination). Animals were without previous antimicrobial therapy and no isolates from the same herds were included repeatedly. The MICs of selected antimicrobials (penicillin, amoxicillin, tetracycline, ceftiofur, enrofloxacin and tulathromycin) were determined by the dilution micromethod according actual CLSI (CLSI, 2013). Results of testing were reported in form MIC_{50} and MIC_{90}.
Results: Based on the evaluation of MIC_{50} and MIC_{90} the most effective antibiotics is amoxicillin with MIC_{50}= 0.06 and MIC_{90} = 0.25 µg/ml. Then followed by enrofloxacin, ceftiofur and penicillin respectively (MIC_{50} = ≤ 0.03 and MIC_{90} = 1 µg/ml; MIC_{50} = ≤ 0.125 and MIC_{90} = 0. 5 µg/ml and MIC_{50} = 0.25 and MIC_{90} = 4 µg/ml). High value of MIC were detected for tetracycline and tulathromycin (MIC_{50} = 2 and MIC_{90} = 64 µg/ml).
According to the available interpretive criteria the highest percentage of resistance was detected for tetracycline (70%), penicillin (20%), enrofloxacin (16. 7%) and tulathromycin (13. 3%).
Conclusion: The prudent use of antimicrobials considers the right choice of the molecule to expect maximum clinical response with the respect to the risk of resistance. Czech isolates of Hps are in general susceptible to amoxicillin and ceftiofur. Taking into consideration the rational use of antimicrobials and ban of cephalosporin's of 3rd and 4rd generation in some important swine producing countries. amoxicillin is considered as a first line treatment. High frequency of resistance to tetracycline was detected and surprisingly 5 isolates were resistant to enrofloxacin. Tulathromycin seems to be clinically not effective for the treatment of systemic infection because of the PK/PD characteristic (low plasma concentration and generally high MIC_{50} and MIC_{90}).

Disclosure of Interest: None Declared

Keywords: antimicrobial resistance, Haemophilus parasuis

Bacteriology and Bacterial Diseases
H.PARASUIS

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Virulence markers of Haemophilus parasuis associated with clinical and pathological outcome in the field

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Introduction: *Haemophilus parasuis* is regularly involved in fibrinous inflammation in pigs, especially relevant for pneumonia, serositis, arthritis, meningitis and pericarditis. As a worst case, SPF herds can suffer from Glässer's disease after acute outbreaks in naïve animals. However, commensalism seems much more common than primary disease and it is hard to differentiate between strains of high and low virulence. The aim of the present survey was to study associations between molecular markers of *Haemophilus parasuis* and the virulence of field isolates.
Materials and Methods: More than 150 strains of *Haemophilus parasuis* were collected from swine herds in Germany (Lower Saxony and Hesse). The severity of disease levels within herds varied from definite cases of commensalism to cases with specific, high degree clinical and pathological symptoms. Individual pigs were dissected and sampled. *Haemophilus parasuis* was cultured and serotyped and 17 putative and potential virulence genes were screened by PCR. The virulence genes were selected from capsular genes (capD), autotransporters (vtaA), outer membrane proteins (ompP2, ompP5), fimbriae (pilF), cytholethal distending toxins (cdt), serine protease-like-proteins (espP2) and bacteriophages (gp36).
Results: Individuals and herds varied significantly in their clinical and pathological outcome, both in a qualitative and quantitative manner. Samples revealed a huge range of strains differing in serotypes and virulence gene patterns. There was no association between serotypes and pathology, but three virulence genes were significantly linked with the severity of disease: ompP2, cdt and espP2a. These genes are well known to be crucial in evading the immune system and thus, for survival in the host. Together, they explained more than 30% of total variation in clinical and pathological symptoms (multiple r = 0.55; p<0,001).
Conclusion: The presented data provide evidence for a significant role of outer membrane protein 2 (ompP2), the cytolethal distending toxin (cdt) and the serine-protease-like-protein 2 (espP2) for the pathogenesis and outcome in *Haemophilus parasuis*-affected pigs in the field. Further investigations and the inclusion of additional molecular factors are needed to efficiently differentiate between *Haemophilus parasuis* strains and to improve future diagnostic opportunities.

Disclosure of Interest: None Declared

Keywords: Diagnostics, Molecular Markers

Bacteriology and Bacterial Diseases
H.PARASUIS

PO-PF3-050

Development of a diagnostic concept for the detection of Haemophilus parasuis infections in pigs in Switzerland

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Introduction: Glässer's disease caused by *Haemophilus (H.) parasuis* leads to significant economic losses due to the need of expensive treatment with antimicrobials, increased mortality in case of acute infection and retarded growth in chronic infections. As *H. parasuis* is a fastidious organism, the isolation of the pathogen and the diagnosis of the disease can be challenging. According to the literature, it would be advisable to sample acutely infected, freshly euthanized pigs and examine the samples bacteriologically as soon as possible. However, Switzerland has a lot of remote areas and the transport of living pigs is rather difficult. Therefore, the aim of this study was to compare two different approaches: 1) to transport pigs suspicious of *H. parasuis* infection alive into a diagnostic facility, where necropsy was immediately followed by bacteriology, and 2) to take samples while performing an on-farm necropsy followed by an overnight-shipment of samples to the next laboratory.
Materials and Methods: This study has been designed for examining 20 pig farms with suspected problems due to an *H. parasuis* infection. In part I of the study up to three acutely infected animals per farm were examined clinically, pathologically and bacteriologically (culture and PCR). Samples included swabs of the serosa, articular cartilage and leptomeninx as well as synovial fluid. When getting evidence of an *H. parasuis* infection, an on-farm necropsy was performed afterwards (part II of the study). During this procedure the same samples as mentioned above were taken, except replacing the swab of the leptomeninx with pericardial fluid, if present. Subsequently, the conditions of an overnight-shipping were simulated.
Results: Thirteen of the 20 farms were tested positive of *H. parasuis* in part I of this study, meaning, that at least one swab of one animal of the farm was positive. In most cases the only positive swab was the one of the leptomeninx – in some cases in addition to another swab. In none of these cases *H. parasuis* was isolated from samples taken during the on-farm necropsies (part II). Moreover, all PCRs were negative (part I + II).
Conclusion: It seems that submitting at least one, but better two live pigs, to the next diagnostic facility is the safest way to diagnose an *H. parasuis* infection. The animals should be showing typical symptoms such as swollen joints, lameness, coughing or fever. After the animals have been euthanized, necropsy including sample taking should be performed straightaway. The samples should include swabs of the serosa, the leptomeninx, although laborious to access, and of the joints, if affected. Immediately after sampling, the swabs should be processed for bacterial testing.

Disclosure of Interest: None Declared

Keywords: diagnostic concept, Glaesser, Haemophilus parasuis

Poster Abstracts

Bacteriology and Bacterial Diseases
H.PARASUIS

PO-PF3-148

The piglet nasal microbiota at weaning and its relation with the development of Glässer's disease

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Introduction: The population of microorganisms living on a particular body site, the microbiota, has been poorly assessed in the respiratory tract in pigs. The nasal mucosa of piglets is early colonized by many bacteria, including *Haemophilus parasuis*, the etiological agent of Glässer's disease. This disease produces economical losses in all the countries with pig production, and the factors influencing its development are not totally understood. Hence, the purpose of this work was to characterize the nasal microbiota composition of piglets, and its possible role in Glässer's disease development.
Materials and Methods: Ten piglets from seven farms from Spain (4 with Glässer's disease and 3 healthy) and three farms from UK (all healthy) were sampled at 3-4 weeks of age, just before weaning. The total DNA extracted from nasal swabs was used to amplify the 16S RNA gene for sequencing in Illumina MiSeq. Sequencing data was quality filtered and analyzed using QIIME software (1.9 version).
Results: The diversity of the nasal microbiota demonstrated to be low in comparison with other body sites, showing a maximum number of OTUs per pig of 1,603, clustered in five phyla. The comparison of the microbiota composition revealed significant differences at various taxonomical levels depending on the farm health status. Health status was associated to higher species richness and diversity, and UK farms demonstrated healthier status profile based on this measurement. When microbiota from Spanish farms with Glässer's disease (GD) was compared with healthy (H) farms, several differential OTUs were found. Importantly, the presence of *Haemophilus* together with other pathogens such as *Streptoccoccus* and *Mycoplasma* were found in higher abundance in GD farms. On the other hand, the OTUs that appeared in higher abundance in H farms were *Oscillospira*, *Prevotella* and *Ruminococcus*, among others.
Conclusion: The composition of the nasal microbiota of healthy piglets was uncovered and different phylotypes were shown to be significantly altered in animals depending on the clinical status of the farm of origin. Several OTUs at genus level were identified over-represented in piglets from healthy farms, indicating their potential as probiotics. In contrast, some OTUs were shown to be increased in GD farms, denoting their potential detrimental role in health. Both group of OTUs deserve further studies to finally confirm the role on Glässer's disease development. In conclusion, the information that provides this work, unraveling the differences among communities in the nasal mucosa, should be taken into consideration for future research related to Glässer's disease control.

Disclosure of Interest: None Declared

Keywords: Glasser disease, Nasal microbiota, pig microbiota

Bacteriology and Bacterial Diseases
H.PARASUIS

PO-PF3-174

Haemophilus parasuis OppA antibody titers indicate Hps infection or vaccination

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Introduction: *Haemophilus parasuis* (Hps) ELISA diagnostic test kits are most commonly detecting antibodies against lipopolysaccharides (LPS), which are present on the outside of the Hps bacteria. However, not all 15 Hps serovars are pathogenic, and Hps LPS can vary between serovars. Furthermore, pigs can be carrier of the Hps bacteria but not undergo an infection. A LPS based ELISA can give a positive S/P ratio in these carrier pigs. This makes the interpretation of results obtained with a LPS based ELISA test kit difficult. Recently BioChek (Reeuwijk, the Netherlands) introduced a Hps ELISA based on the permease A (OppA) protein. OppA is a transmembrane protein, that resides inside the Hps bacteria. Antibodies against OppA are only detected after Hps infection or vaccination. The BioChek Hps OppA ELISA was tested for its suitability.
Materials and Methods: The BioChek Hps OppA ELISA was tested on sera from healthy finishing pigs with no clinical signs of a Hps infection, on sera from animals suffering from a Hps infection and on sera from animals that were vaccinated against Hps.
Results: The immune dominant OppA protein is presented by macrophages to the immune system during Hps infection or after Hps vaccination only, healthy carrier animals will show no serological response to Hps OppA. A set of 25 samples taken from finishing pigs of different age groups was tested in both a Hps LPS and the BioChek Hps OppA ELISA. No clinical signs of Hps were present in the herd. In the LPS ELISA a positive S/P ration was found in 6 out of 25 samples while all samples were negative when tested in the BioChek Hps OppA ELISA. In a similar study the conventional LPS ELISA detected Hps antibodies in 13 out of 50 samples and the OppA ELISA detected antibodies in 1 sample only. In a case with clinical Hps and involving 90 finishing pigs, the LPS ELISA detected antibodies in 71 out of 90 samples while the OppA ELISA detected antibodies in 7 out of 90 samples, which is a reflection of the low morbidity of Hps in a herd. In vaccinated herds OppA antibodies can be detected in animals starting from 2 weeks after vaccination. At 10 weeks of age and after 2 vaccinations, 100% of the animals were positive in the BioChek Hps OppA ELISA.
Conclusion: The LPS based ELISA will not discriminate between healthy carriers and animals suffering from a Hps infection. All Hps serovars and all vaccine induced antibodies will be detected in the BioChek Hps OppA ELISA. With the low (<10%) morbidity of Hps in conventional herds, it is important to collect sufficient samples (a minimum of 20).

Disclosure of Interest: None Declared

Keywords: BioChek , ELISA , Hps

Bacteriology and Bacterial Diseases
LAWSONIA

PO-PF3-034

Infection dynamics of intestinal pathogens in batches of non-medicated diarrheic nursery pigs

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Introduction: It has been established that pooled faecal pen floor samples (PFP) tested by quantitative PCR (qPCR) can be used as at diagnostic tool to assess the average excretion level in groups of pigs. In Denmark a commercial qPCR test for four important intestinal pathogens in nursery pigs (*E. coli* F4 (F4) and F18 (F18), *Lawsonia intracellularis* (LI) and *Brachyspira pilosicoli* (PILO)) has been developed. The aim of this study was to investigate the infection dynamics of the four mentioned pathogens and assess the diarrhoea prevalence in batches of non-medicated nursery pigs 14-35 days after weaning.
Materials and Methods: A longitudinal study was performed in three production farms in Denmark. PFP samples were collected weekly from pens containing nursery pigs (day 14, 21, 28 and 35 post weaning). The samples were examined for F4, F18, LI and PILO by qPCR testing. Furthermore, faecal samples obtained from pigs was clinically assessed as diarrhoeic or not and the diarrhoea prevalence at pen level was calculated. Pens subjected to antibiotic batch medication during the study were excluded.
Results: A total of 78 pens were included in the start of the study at day 14. Due to antibiotic batch medication 58, 31, and 13 pens were included for analysis at day 21, 28 and 35 after weaning respectively. The prevalence of pathogens over time was F4 = 0.03, 0.02, 0, 0; F18 = 0.51, 0.45, 0.13, 0; LI = 0.19, 0.40, 0.74, 0.92 and PILO = 0.05, 0.10, 0.16, 0.15. Diarrhoea prevalence for the individual pigs was 0.12, 0.28, 0.26 and 0.39. In the positive pen samples, the excretion level was increasing during the study-period for all four pathogens, the lowest at day 14 and the highest at day 35. The infection patterns of LI were the same in the three study herds but variation was observed with F4, F18 and PILO. F4 was only found in 2 of 3 herds. F18 was found in all three herds at day 14 and 21 but was only found at day 28 in one herd. PILO was also found in all three herds, but varied over time.
Conclusion: The study showed that there was similar occurrence of pathogens at several time points. In all herds F18 and LI was the most frequently detected pathogen. While F18 was the dominant pathogen in the beginning LI was dominating in the end of the study period. PILO was detected at decreasing frequency over time. F4 was a rare finding in this study. This study also showed that the total pathogen excretion level of positive samples and the diarrhoea prevalence increased over time. Due to antibiotic treatment the sample size decreased over time from 78 pens to 13 which may have influence on the results.

Disclosure of Interest: None Declared

Keywords: Diarrhoea, Lawsonia intracellularis, nursery pigs

Bacteriology and Bacterial Diseases
LAWSONIA

PO-PF3-078

Significant reduction of flock medication in weaners after vaccination against Lawsonia intracellularis

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Introduction: In Denmark, a political goal has been set to reduce the consumption of antibiotics (AB) in the Danish pig production by 15% before 2018. Treatments of intestinal disorders in weaners are responsible for a majority of the AB consumption and therefore, prophylactic measures to prevent intestinal disorders are necessary to achieve this reduction.
The herd presented here works intensively with prevention in order to lower their AB consumption, and vaccination against Lawsonia intracellularis was implemented as an aid in achieving this goal.
Materials and Methods: The project herd is a typical Danish sow herd with 500 sows, weaning piglets to separate barns on the same site. At approximately 30 kg live weight, pigs are sold for finishing. From November 1st 2014, the pigs were vaccinated against Lawsonia intracellularis in the first week after weaning (Enterisol Ileitis, Boehringer Ingelheim). The vaccine was administered in through with a dose of 2 ml per pig.
Data for antibiotic prescriptions before and after introduction of the vaccine was collected from the Danish Vetstat database. Production figures were obtained from efficacy reports prepared by the local agricultural consultants. Statistical analysis comparing antibiotic use without and with vaccination was made with Mann Whitneys U-test, with p=0.05 as level of significance.
Results: After introduction of the vaccine, the antibiotic use, measured as Animal Daily Doses, ADD, decreased significantly, from a monthly mean of 8.1 before vaccination to 3.7 ADD after vaccination ($p<0.02$). The main reduction was seen in prescriptions for intestinal disorders. The actual amount of active compound used for treatment of intestinal disorders decreased with 63% or 8.9 kg in the first year with vaccination compared to the previous year ($p<0.02$). Production figures were influenced not only by the introduction of the vaccination against Lawsonia intracellularis, but also by an increase of the mean weaning age with 3.5 days, which lead to sale of pigs at a lower weight in order to keep a consistent pig supply for the finishing herds. The sale of lighter pigs reduced ADG in the nursery from 495 to 481 g/day. The mortality stayed at 2.4% after vaccination, and FCR was improved from 1.89 to 1.73 FE/kg gain.
Conclusion: Vaccination against Lawsonia intracellularis made it possible to produce pigs for finishing with 1/3 of the AB that was used before vaccination. Piglet mortality stayed at the same level as with the high AB consumption, indicating that the significant reduction of AB use did not have a negative influence on piglet health. The productivity was at least as good as when the herd used more antibiotics.

Disclosure of Interest: N. R. Weber: None Declared, S. Mikkelsen: None Declared, J. Bagger Conflict with: Empoyee at Boehringer Ingelheim, H. Bak Conflict with: Employee at Boehringer Ingelheim

Keywords: Lawsonia vaccination, reduce antimicrobials

Poster Abstracts

Bacteriology and Bacterial Diseases
LAWSONIA

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Effect of oral doxycycline and tylosine treatment on faecal Lawsonia intracellularis excretion

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Introduction: *Lawsonia intracellularis* (LI) is an important enteric pathogen in nursery pigs worldwide. High excretion level of LI (> 10^6 LI bacteria/g faeces) is correlated to histopathological lesions of proliferative enteropathy and decrease of daily weight gain. In Denmark doxycycline and tylosine are the most commonly used antimicrobial oral treatments for LI in nursery pigs. A typical treatment period is 5 days. The purpose of this study was to compare the effect of doxycycline and tylosine treatment on faecal excretion of LI two days after treatment.
Materials and Methods: A total of 65 pens from batches of nursery pigs in three herds 14 to 28 days post weaning, was randomly assigned and treated for 5 days with 12.5 mg per kilo bwt. doxycycline (DOX) or 5 days with 7.5 mg per kilo bwt. tylosine (TYL) via water through. Treatments was initiated randomly at day 14, 21 or 28 post weaning independently of diarrhoea status of the pigs. Pooled faecal pen samples was collected at the day of initiation of treatment and two days after last day of treatment and analysed by qPCR for LI with a lower detection limit of $2x10^3$ LI bacteria/g faeces. The association between type of treatment and prevalence of LI positive faecal samples after treatment and prevalence of LI at high level (>10^6 LI bacteria/g faeces) after treatment were tested by chi^2 test.
Results: At the day of initiation of treatment, LI was detected in 12 of 33 pens treated with DOX (mean excretion = $10^{6.6}$ LI bacteria/g faeces) and in 14 of 32 pens treated with TYL (mean excretion = $10^{6.5}$ LI bacteria/g faeces). At the second sample two days after treatment LI was detected in 7 of 33 pens treated with DOX (mean excretion = $10^{5.2}$ LI bacteria/g faeces) and in 18 of 32 pens treated with TYL (mean excretion = $10^{6.7}$ LI bacteria/g faeces). There was an association (p = 0.008) between type of antimicrobial compound and detection of *L. intracellularis* (>$2x10^3$ bacteria/g faeces) two days after treatment with an odds ratio of 4.78 (CI95 % 1.61-14.18) in pens treated with TYL compared to pens treated with DOX. There was also an association (p = 0.003) between type of antimicrobial compound and detection of high level *L. intracellularis* (>10^6 bacteria/g faeces) two days after treatment with an odds ratio of 10.67 (CI95 % 1.25-91.13) in pens treated with TYL compared to pens treated with DOX.
Conclusion: The results of this study showed that 5 days treatment with DOX was more effective in reducing excretion levels of LI then 5 days treatment with TYL. Significant higher levels of LI that correlates to gross lesions of proliferative enteropathy and decrease of daily weight gain were found in pens treated with TYL.

Disclosure of Interest: None Declared

Keywords: ANTIMICROBIAL, Lawsonia intracellularis, nursery pigs

Bacteriology and Bacterial Diseases
LAWSONIA

PO-PF3-232

Comparison of Aivlosin® and Denagard® in the Treatment of Porcine Proliferative Enteropathy

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Introduction: Tylvalosin (TVN), a 2nd generation macrolide antibiotic, is the active ingredient in Aivlosin®. Aivlosin® is authorised throughout the European Union and other international markets for the treatment of respiratory and enteric disease in swine. The objective of this study was to compare tylvalosin with tiamulin (Denagard®), in the treatment of porcine proliferative enteropathy (PPE) caused by *Lawsonia intracellularis*.
Materials and Methods: The study was conducted in a commercial wean to finish unit in Ireland. Clinical signs and diagnostic tests confirmed the presence of *Lawsonia intracellularis* and *Mycoplasma hyopneumoniae* in the herd. Two groups of 780 pigs, each weighing *ca* 25 kg, were administered either Aivlosin® 42.5 mg/g Premix (4.25 mg tylvalosin/kg bodyweight for ten consecutive days) or Denagard® 2% w/w Premix (7.5 mg tiamulin/kg bodyweight for 21 consecutive days) in wet feed. The study continued until the pigs were sent to slaughter aged 26 weeks.

Group and individual data from 240 pigs in each group were collected. Daily observations, including mortality were recorded throughout. Live bodyweight data were collected on the Study Days 0 (start of treatment), 21 and at slaughter. Deadweight at slaughter was also measured. Faecal consistency was scored on Study Days 0 and 21. Lung scores at slaughter were determined using the Goodwin Method
Results: Clinical scores for faecal consistency were lower for Aivlosin® but not statistically different from Denagard®. Average lung scores were low for both Aivlosin® and Denagard®, at 1.3% and 0.6%, respectively. Average bodyweight on Study Day 21 plus live weight and deadweight at slaughter for pigs treated with Aivlosin® were significantly higher (p<0.01%) than those treated with Denagard®. Average daily gain for the Aivlosin® and Denagard® treated groups, was 840.7 g and 753.7 g, respectively (p<0.01%). Using a financial comparison model, the advantage of using Aivlosin® over Denagard® in this study was estimated to be £3.92 (5.33€) per pig started
Conclusion: Aivlosin® 42.5 mg/g Premix and Denagard® 2% w/w Premix when used in the treatment of naturally occurring PPE both performed well clinically. Aivlosin® 42.5 mg/g Premix however is administered at a lower dose for a shorter duration and provides a commercial advantage

Disclosure of Interest: P. Kirwan: None Declared, J. Mora Conflict with: Eco Animal Health Ltd., M. Tigges Conflict with: Eco Animal Health Ltd

Keywords: Porcine Proliferative Enteropathy, Swine

Bacteriology and Bacterial Diseases
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Protection of piglets against Lawsonia intracellularis challenge by vaccination with a single dose of a killed, injectable vaccine

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Introduction: Until recently, few solutions existed against ileitis in swine caused by *Lawsonia intracellularis (Li)*: either a live attenuated vaccine administered orally, or antibiotics. This study sought to demonstrate the ability of an inactivated bacterin, administered as a single intramuscular (IM) dose, to protect against experimental challenge.
Materials and Methods: Three week old, weaned pigs with low anti-*Li* antibody levels were used for the study. Groups of 15 pigs each were vaccinated with a 2 mL IM dose of inactivated *Li* bacterin, formulated in an oil-in-water adjuvant, or a placebo vaccine. A positive control group of 15 pigs was orally drenched with a commercially available live attenuated vaccine, according to the manufacturer's instructions. Two unvaccinated littermates were commingled within each group, which served as sentinels of lateral *Li* infection until the time of the challenge.
At 10 weeks of age, serum samples were collected to measure antibody responses to the vaccination. At this time, the sentinels were necropsied to confirm the absence of *Li* infection, and the vaccinated, placebo and positive control groups were orally challenged with a gut homogenate containing virulent *Li.* Following challenge, all pigs were monitored for clinical signs of ileitis and body weights, and fecal samples were collected.
The challenged pigs were sacrificed at 21 days post-challenge. In the necropsied pigs, incidence and severity of ileitis were evaluated by gross and microscopic observation of ileal tissues. Colonization was examined by immunohistochemistry, and qPCR of ileum scrapings. Fecal shedding was monitored by *Li* specific qPCR.
Results: No evidence of *Li* infection was noted in sentinel pigs. All pigs treated with the *Li* bacterin had antibodies against *Li* at the time of the challenge, while all placebo animals and the positive controls were negative. Clinical signs of ileitis were reduced in the test group as compared to the two control groups. At 21 days post challenge, gross and microscopic lesions of ileitis as well as *Li* colonization were markedly reduced in bacterin-vaccinated pigs compared to both control groups. Bacterin-vaccinated pigs shed significantly less *Li* compared to the placebo group as well as the commercially available live vaccine. Further, daily weight gain as a result of the challenge was markedly improved using the inactivated bacterin vaccine.
Conclusion: This study confirmed the ability of a killed bacterin to protect weaned pigs against experimental challenge with *Li*, resulting in reduced disease and fecal shedding, and improved weight gain. Interestingly, no evidence of vaccine response or protection against *Li* challenge was demonstrated in the live vaccine control group.

Disclosure of Interest: F. Roerink Conflict with: Merck Animal Health, C. Morgan Conflict with: Merck Animal Health, R. Segers Conflict with: Merck Animal Health, E. Strait: None Declared

Keywords: Lawsonia vaccination

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Efficacy of Porcilis® Ileitis under field conditions, evaluated with an experimental challenge model

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Introduction: Ileitis caused by *Lawsonia intracellularis (Li)* continues to be a major problem in swine production systems worldwide. A killed, injectable vaccine is now commercially available in the United States. Porcilis® Ileitis aids in the control of ileitis caused by *Li*, aids in the reduction of colonization by *Li* and aids in the reduction of duration of fecal shedding. Duration of immunity for at least 20 weeks has been demonstrated. This study sought to investigate the efficacy of Porcilis Ileitis under field conditions.
Materials and Methods: Pigs were sourced from a commercial, farrow-to-finish farm, which farrowed 50 litters per week. Forty pigs per group were IM vaccinated at 3-weeks of age with 2 mL of Porcilis Ileitis, or with saline. Another group of 5 littermates were maintained as sentinels.
At weaning, pigs were moved to an isolated facility where they were housed in a single air-space. Five weeks after vaccination, the vaccinated and control groups were orally challenged with a gut homogenate containing virulent *Li.* At this time, the sentinels were necropsied to confirm the absence of *Li* infection.
A subset of vaccinates and control pigs were sacrificed at 21 days post-challenge, while another subset of pigs were fecal monitored three times weekly for *Li* shedding until 52 days post-challenge. In the pigs necropsied at 21 days after challenge, incidence and severity of ileitis were evaluated by gross and microscopic observation of ileal tissues. Colonization was examined by immunohistochemistry, and qPCR of ileum scrapings. Fecal shedding was monitored by *Li* specific qPCR. Serum samples were collected throughout the study, to measure antibody responses by ELISA.
Results: At the time of vaccination, all pigs were free of anti-*Li* antibodies. Vaccination with Porcilis Ileitis resulted in seroconversion in 53% of the pigs by 3 weeks post vaccination. No systemic or injection site adverse reactions were observed in any of the pigs following vaccination. No evidence of *Li* infection was noted in the sentinel pigs. At 21 days following the challenge, gross and microscopic lesions of ileitis were markedly reduced in vaccinated pigs, compared to controls. The same was observed for the immunohistochemistry and qPCR results of the ileum scrapings.
Around the peak time of fecal shedding, vaccinated pigs shed significantly less *Li* compared to the controls. This difference was on average 10 Ct, which corresponds to a 3 $\log_{10}$ reduction.
Conclusion: This study confirmed a significant reduction in disease, colonization and shedding due to *Li*, when 3-week old pigs were vaccinated with Porcilis Ileitis under field conditions.

Disclosure of Interest: F. Roerink Conflict with: Merck Animal Health, C. Morgan Conflict with: Merck Animal Health, S. Knetter Conflict with: Merck Animal Health, R. Segers Conflict with: Merck Animal Health, E. Strait Conflict with: Merck Animal Health

Keywords: Lawsonia vaccination

Poster Abstracts

Bacteriology and Bacterial Diseases
LAWSONIA

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Improvement of production results in a Korean farrow-to-finish farm with PHE after implementation of Enterisol Ileitis vaccination.

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Introduction: Ileitis is an enteric disease caused by Lawsonia intracellularis. Despite Ileitis being common in the swine industry farm, managers are not familiar with it. Most of the Ileitis cases are mild or subclinical with no obvious clinical signs. Ileitis is often not treated in the farm until there is a severe or haemorrhagic diarrhea. But the subclinical type of Ileitis in the grower-finisher house can also cause significant losses. This case report describes the benefits of implementing oral live vaccination against Ileitis of clinical type, first as mass vaccination of pigs 3 to 15 weeks of age, followed by routing vaccination at 3 weeks of age.
Materials and Methods: This study was conducted in a one-site system of 150 sow farm. From March of 2013, a continuous diarrhea in nursery and haemorrhagic diarrhea in grow-finisher began to appear. Mortality increased and many pigs had clinical signs of wasting and lethargy. Feces samples from pigs were used to detect enteric pathogens in the nursery, grower and finisher house. Samples were tested by PCR for presence of Lawsonia intracellularis, Swine dysentery, and Salmonella. First, Tiamulin was used to control diarrhea but symptoms did not completely disappear. Vaccination of pigs with Enterisol Ileitis was implemented as a second measure. Initially all pigs between 3 and 15 weeks of age were vaccinated against Ileitis in the nursery and grower house, followed by routine vaccination at 3 weeks of age. Five days before and after vaccination, no antibiotics were applied to protect vaccine efficacy. Mortality and slaughter data were compared before (Apr.-July 2013), in transition (Aug. – Oct. 2013) and after vaccination (Nov.-Dec. 2013).
Results: Lawsonia intracellularis was detected by PCR test in the feces samples. After implementing vaccination with Enterisol Ileitis mortality decreased from 12.5% to 6.6% in the grower-finisher house and from 2.3% to 1.9% in the nursery house. The average age of slaughter was reduced from 210 days of age to 190 days of age and the weight at slaughter increased from 108 kg to 114 kg after implementing Ileitis vaccination.
Conclusion: After the implementation of Enterisol Ileitis, not only the mortality but also other performance parameters like average age and average weight at slaughter were improved clearly. The performance improvement in the transition period demonstrated that even older pigs up to 15 weeks of age can benefit from vaccination. Compared to the use of antibiotics only, vaccination used in addition is much more effective to control Ileitis

Disclosure of Interest: None Declared

Keywords: Enterisol ileitis, Lawsonia intracellularis, PHE

Bacteriology and Bacterial Diseases
LAWSONIA

PO-PF3-233

Vaccination against Lawsonia Intracellularis reduces antibiotic consumption in weaners and finishers

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Introduction: Reduction of the antibiotic consumption pigs is subject of political awareness, and methods to reduce the consumption are in great demand. Vaccination against ileitis can be one of the methods.
Materials and Methods: From the Danish Vetstat database, a search was made for one year prescriptions of Zinc to select herds with weaners, and on prescription of ileitis vaccine (Enterisol Ileitis, Boehringer Ingelheim). From the data extract, the herds that with prescription of Enterisol corresponding to regular vaccination of piglets were identified. As the non-vaccinated control group, a similar number of non-vaccinated herds were selected in order of appearance. Finishing herds growing pigs from the selected weaner herds were identified through pig movements recorded in the CHR register.

From every herd, a 9 month average for prescription of antibiotics was recorded from the end of the period with vaccination. The antibiotic consumption was recorded as ADD (Animal Daily Doses) per 100 pigs per day. For the weaners, the specific prescriptions for intestinal disorders during a full year were also extracted, and the amount of active compound prescribed for each group was calculated. The antibiotic consumption in vaccinated and non-vaccinated herds was compared with Mann-Whitneys U-test, with p=0.05 as level of significance.
Results: Totally, 26 weaner herds were included as the vaccination positive group and thus, 26 non-vaccinated herds were included as well. The herds in the two groups were comparable according to size and health status. Finisher data were available from 24 vaccinated herds and from 18 non-vaccinated herds due to export of pigs from the remaining herds.

The 9 month average of antibiotic consumption was 10.1 ADD/100 pigs/day in vaccinated weaner herds and 11.4 ADD/100 pigs/day in non-vaccinated herds. This gave an annual reduction of 2,3 kg active compound (e.g. tetracycline) per vaccinated weaner herd compared to non-vaccinates. In the finishing herds, the 9 month average was 1.95 ADD/100 pigs per day in vaccinated herds and 1.99 ADD/100 pigs/day in non-vaccinated herds. None of the differences were statistically significant.
Conclusion: The present study shows that herds with vaccination against ileitis have a lower consumption of antibiotics than non-vaccinating herds. The study design did not include diagnostics, and the data set might be biased, because Lawsonia Intracellularis might not be present in some of the control herds. Furthermore, more exporting herds were present in the control group, giving only a small sample size for non-vaccinated finishing herds. In the individual herd infected with Lawsonia Intracellularis, the reduction can be expected to be on a higher level.

Disclosure of Interest: M. Agerley: None Declared, H. Bak Conflict with: Vaccine company

Keywords: database study, reduce antimicrobials, Vaccination

Bacteriology and Bacterial Diseases
LAWSONIA

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Efficacy of five oxytetracycline treatment regimens for Lawsonia intracellularis infection in nursery pigs

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Introduction: Treatment with antimicrobials is widely used in pig production for the control of gastrointestinal infections. *Lawsonia intracellularis* (LI) causes enteritis in pigs older than six weeks of age and is a common reason for treatment. The World Organisation for Animal Health recommends assessment of therapeutic efficacy in clinical trials examining both dosing regimens and route of administration. This study describes the efficacy of five oxytetracycline (OTC) treatment regimens for LI infection in nursery pigs measured as faecal shedding of LI, faecal dry matter (FDM) content and average daily weight gain (ADG).
Materials and Methods: A randomised clinical trial was carried out in 4 Danish nursery herds involving 63 batches and 939 pigs. Five OTC treatment regimens for LI infection were randomly allocated and implemented at batch level and with OCT administered for 5 days. The 5 treatment regimens were: Oral (PO) batch medication of all pigs in a batch through drinking water in a dose of either 20, 10 or 5mg OTC/kg pig/day, or PO treatment of diarrhoeic pens only by water troughs with 10mg OTC/kg pig/day or intramuscular (IM) treatment of individual diarrhoeic pigs only by injection of 10mg OTC/kg pig/day. Each treatment was repeated in 3 or 4 batches in each herd. The efficacy was evaluated for faecal shedding of LI, FDM and ADG after treatment using mixed models with herd and batch as random effects.
Results: Treatments involving PO batch medication of all pigs in a batch, all reduced diarrhoea and LI shedding after treatment compared to before treatment (p<0.05). Batches with 5mg OTC treatment tended to have more watery faeces (p=0.06) and 5.5 times higher odds of LI shedding after treatment (p<0.05), compared to doses of 10 and 20mg OTC. Treatments involving PO treatment of diarrhoeic pens only by water troughs with 10mg OTC/kg pig/day also reduced diarrhoea and LI shedding after treatment (p<0.01), whereas IM treatment of diarrhoeic pigs only, did not reduce the number of pigs with LI shedding at batch level after treatment (p=0.4). Batches with pen-wise or individual treatments, both had 11 times higher odds of LI shedding after treatment (p<0.05) compared to batches with PO treatment of all pigs in drinking water. No significant difference in ADG was observed among treatments. A high LI infection level was correlated to a decreased ADG (p<0.01).
Conclusion: The lowest risk of LI shedding after OTC treatment was achieved by oral treatment of all pigs with 10mg OTC/kg pig/day, when compared to both OTC treatment with a lower dose and OTC treatment of a smaller number of animals. No overall effect of treatment regimen on ADG was demonstrated.

Disclosure of Interest: None Declared

Keywords: antimicrobial usage, Lawsonia intracellularis, treatment regimen

Bacteriology and Bacterial Diseases
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Case review: Adaptation of oral ileitis vaccination to the health status of the piglets

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Introduction: Damage caused by *Lawsonia intracellularis* (Li) infection can be controlled by oral ileitis vaccination. Vaccination in general, is advised in clinically healthy animals.
The objective of this case review is to show factors that may influence the effect of oral ileitis vaccination.
Materials and Methods: A 500 head sow farm sells 9-10 weeks old piglets to two finishing farms. At both finishing farms, pigs showed symptoms of growth retardation and paleness which was diagnosed by necropsy as PIA (GD Animal Health Deventer). Oral ileitis vaccination by drench (Enterisol® Ileitis) was started at 5-7 weeks of age.
At 4 months after the start of the ileitis vaccination in both finishing farms the clinical situation had not improved. Diagnosis was again made by necropsy: PIA.
A second visit to the sow farm revealed diarrhea at all ages from weaning until transport to the finishing farms. Immediately the ileitis vaccination was rescheduled to 3 weeks of age.
Results: Before vaccination at 9 weeks of age in 10 serum samples no Li antibodies were found (bioScreen ELISA).
After vaccination necropsies of piglets at 7 weeks of age showed intestinal problems. Histology of small and large intestines showed proliferative enteritis and some crypt abscesses. Additional tests were negative for a variety of pathogens. Fecal samples of piglets 5 and 7 weeks of age only tested positive for E.coli. Drinking water samples were tested as polluted by enterococci and coli like species.
Conclusion: Starting ileitis vaccination at 5-7 weeks of age was advised as Li serum antibody tests showed Li-infection not before the age of 7 weeks.
The necropsies in piglets 7 weeks of age provided no diagnosis of infectious disease. Drinking water quality was poor. For improvement of the intestinal health, first the water quality had to be good.
In this case the advice to vaccinate only clinically healthy animals was ignored. Diarrhea is a clinical sign that is often not very obvious and can easily be missed. The farmer at the sow farm regarded 'some diarrhea' not as clinical symptom of sickness.
Rescheduling the ileitis vaccination to an age of 3 weeks, before weaning when there was no clinical sign of diarrhea, and cleaning the drinking water system had an immediate good effect. There were no more complaints in the finishers.
Meanwhile, the farmer has changed the ileitis vaccination to application by the drinking water system, in clinically healthy piglets at 7 weeks of age. With continuous good results in the finishers.
In this case the conclusion is that, when applying oral vaccination, one has pay good attention to intestinal health issues like diarrhea around the age of vaccination.

Disclosure of Interest: None Declared

Keywords: Diarrhoea, Lawsonia, Vaccination

Poster Abstracts

Bacteriology and Bacterial Diseases
LAWSONIA

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Case study: Cost effective oral ileitis vaccination in already well performing finishers

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Introduction: *Lawsonia intracelluaris* (Li) is a bacteria well known to be wide spread in pig populations all over the world. At the finishing site involved in the study, Li infection was suspected because of sporadic mortality of animals with enteric bleeding. No negative effect on technical performance data was recognized. The goal of this study was to evaluate a possible return on investment of oral ileitis vaccination at the finishing site.
Materials and Methods: The study took place at a Dutch finishing site, 4300 places, continuous flow, 175 pigs per room (all in). By cross sectional serum testing (bioScreen ELISA) Li infection was confirmed and an indication of the moment of Li infection was found.
During a period of six weeks, alternating per room, pigs were vaccinated (EI) or not vaccinated (Control). Males and females were almost equally distributed among the groups. Oral ileitis vaccination (Enterisol Ileitis®) in the drinking water was started four days after the pigs entered the finishing site. During the study no antibiotic group treatments with an indication for Li infection were given.
Date and weight of the piglets were registered at arrival in the finishing site. When the first animals in a room were marketed for slaughter, all the remaining animals were weighed and the average daily gain per room was calculated from recalculation of the slaughter weights to live weights and from the measured live weights. Mortality was registered per room.
Results: At 22 weeks of age Li antibodies were detected shortly before and by the end of the study.
Both study groups consisted of 6 rooms, about 1000 pigs per group. Compared to the Control group the EI group had: an increase in average daily gain (841 versus 854 gram/ day) and a decrease in mortality (3.1% versus 2.2%)
Conclusion: The serology results showed the moment of Li infection on a batch level in the second half of the finishing period. This gave the time-window to vaccinate the piglets shortly after arrival at the finishing site. From the control group we excluded 92 'poor doers' that were regrouped in another room and as a result we missed the slaughter date and slaughter weights. The EI group had no regrouped animals.
Oral ileitis vaccination at this farm turned out to be cost effective. We believe that if the 'poor doers' would have been included in the study, the benefit would have been better. As feed prices have gone up over time, the benefit with the actual (increased) feed prices (Dec.2015) would have been better
The pig owner first stopped the vaccination program after the study, but restarted the oral ileitis vaccination 6 months later and continues doing so.

Disclosure of Interest: None Declared

Keywords: lawsonia, Subclinical, Vaccination

Bacteriology and Bacterial Diseases
LAWSONIA

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Detection of bacterial enteric pathogens in fattener pigs. A cross-sectional study in eight farrow-to-finish herds in Argentina

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Introduction: *Lawsonia intracellularis* (LI), *Brachyspira hyodysenteriae* (BH), *Brachyspira pilosicoli* (BP) and *Salmonella enterica* (SE) are important enteric bacterial infections in fattener pigs. Little information related to the field dynamic of these infections in Argentina is available. The objective of this work was to investigate the prevalence and association of LI, BH, BP and SE with the use of antibiotics, age of detection and presence of diarrhea.
Materials and Methods: A total of 10 fecal samples of pigs with and without diarrhea of 8, 11, 14, 17, 20 and 24 weeks-old were collected from 8 farrow-to-finish herds. Information about use of antibiotics was obtained. DNA was extracted and was analyzed by multiplex PCR assay to identify LI, BH and BP. For SE, samples were cultures using standard methods. In each farm and age groups the percentages of pigs positive by PCR were calculated. Correlation analysis was applied between positive pigs with and without diarrhea. Logistic regression was performed for each agent to calculate the risk age and the presence of diarrhea.
Results: *Lawsonia intracellularis* and SE were found in pigs whit and without diarrhea in 8 and 5 farms respectively. There was association between LI shedding and presence of diarrhea (OR 2.3;p<0.05). Also, BH was found in pigs at 11 and 14 weeks-old in two herds, while BP was not detected. No significant associations were found for BH and SE. Positive correlation between LI positive pigs with and without diarrhea was obtained (r=0.71;p<0.05). Pigs age was a risk factor for LI and SE detection (p<0.05). The OR`s by age for LI were: 14 OR=7.46; 17 OR=12.3; 20 OR=30.8; 24 OR=47.7 and SE: 20 OR=4.6; 24 OR=5.5. Four serotypes of *Salmonella* were identified: *S. Tennesse, S. Derby, S. Typhimurium* and *S. Javiana*. All farms removed antibiotics from 17 to 20 week-old pigs
Conclusion: -Detection of LI increases 2.3 times the chance to be diarrheic. However, correlation between pigs with and without diarrhea showed a subclinical infection. Hence, the economic impact of subclinical endemic LI may be underestimated in absence of complementary diagnostic.
-No significant associations were found between SE and diarrhea. It could be associated with the detection of non pathogenic serotypes with the exception of *S. Typhimurium*.
-The low percentage of detection of BH and the absence of BP may be explained by the low prevalence of both pathogens in Argentina.
-The risk of detection LI and SE increase with the age. Moreover, when feed antibiotics were removed, the OR`s were higher. These results suggest that inhibition of bacterial growth by antibiotics may play a role in the clinical expression of enteric infection.

Disclosure of Interest: None Declared

Keywords: enteropathogens, grower finish herds,, swine

Bacteriology and Bacterial Diseases
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Evaluating the Use of an EnviroBootie to Detect Lawsonia and Salmonella from Known Positive Concrete Surfaces

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Introduction: An EnviroBootie™ (Hardy Diagnostics) has been described in the poultry industry as a surveillance tool to identify the presence of *Salmonella* enteritidis following an FDA mandate for egg-layers. It is our intention to demonstrate the feasibility of a cotton mesh booty soaked in neutralizing broth as a surveillance tool for demonstrating the presence of enteric organisms in the growing pig environment. The objective of this pilot study was to evaluate the ability to detect *Lawsonia intracellularis*, *Salmonella* choleraesuis and *Salmonella* Typhimurium in the environment utilizing the environmental bootie.
Materials and Methods: Utilizing a 20ft x 60ft solid concrete slab (drive way), 3 blocks of 3 collection patterns (9 blocks total) were created. Each treatment block measured 10ft x 12ft. The contents of two 100 dose bottles of vaccine were misted over the concrete surfaces (1x100 dose bottle of Enterisol® Ileitis and 1x100 dose bottle of Enterisol Salmonella T/C®; Boehringer Ingelheim Vetmedica, Inc.). Following vaccine application, the technician intentionally walked each of nine 10ft x 12ft squares so that 3 squares were walked in a cross pattern (squares: 1x, 2x, 3x) and 3 squares in a circle pattern (squares: 1c, 2c, 3c) and 3 squares by shuffling feet (squares 1s, 2s, 3s). *Note: walking pattern consisted of Heel-to-Toe steps across treated surface. The envirobooties were removed from the technician and placed into individual plastic bags (2/bag) containing 50ml of DE neutral broth and identified by treatment number. All samples were submitted to BI HMC for Lawsonia PCR and ISU-VDL for Salmonella culture. Two booties were placed into a plastic bag with neutralizing broth without exposure to concrete surface to serve as a negative control.
Results: All concrete samples were positive for Salmonella culture at ISU-VDL and all concrete samples were PCR positive for Lawsonia except Rep 3 when shuffling feet. The negative control sample was negative for Lawsonia PCR but positive via Salmonella culture.
Conclusion: The environmental bootie appears to be a valid and highly sensitive method for detecting Lawsonia via PCR and Salmonella via culture on a concrete surface independent of walking pattern utilized. However, walking in a circle or cross pattern across the concrete surface was 100% effective in detecting the known Salmonella or Lawsonia applied to the concrete surface. A positive Salmonella culture of the control sample (no concrete contact) suggests strict attention will need to be given to handling and packing of samples for shipping.

Disclosure of Interest: None Declared

Keywords: EnviroBootie, Lawsonia, Salmonella

Bacteriology and Bacterial Diseases
LAWSONIA

PO-PF3-180

Effect of ileitis oral vaccination against Lawsonia intracellularis on performance improvement in a Spanish company

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Introduction: *Lawsonia intracellularis* (L.i.) is the causative agent of porcine proliferative enteropathy (PPE). PPE is a relevant economic enteric disease that causes diarrhea and reduces weight gain in growing pigs (1). The subclinical form produces as well a negative impact on performance and farm economics. L.i is endemic in most of the Spanish farms (2). The aim of this study was to evaluate the efficacy of Enterisol® Ileitis (Boehringer Ingelheim Vetmedica GmbH) in a Spanish commercial company.
Materials and Methods: This study was conducted in a 450 sows farrow to feeder (20kilos) farm located in the eastern region of Spain.The farm is negative for PRRS. Pigs at fattening were suffering subclinical ileitis and L.i. infection was confirmed by ELISA (IgG). A total of 23100 fattening pigs were included in the study (10250 non-vaccinated and 12850 vaccinated with the oral nonvirulent live vaccine Enterisol® Ileitis (Boehringer Ingelheim Vetmedica GmbH). Thus, the whole production of 2013(13 batches) vs 2014(16 batches). The piglets were orally vaccinated via drinking water at entry in the finishing unit using Thiosulfate Blue (Boehringer Ingelheim Vetmedica GmbH) as stabilizer. All the animals were raised under similar conditions same fattening units, water and feed supply. The parameters recorded were: feed conversion rate (FCR), mortality rate (%) and gastro-intestinal antibiotics costs (€). Data has been analysed using ANOVA with SPSS v.15.0 (SPSS Inc., Chicago, IL, USA) software. The Benefits cost ratio was calculated by using BECAL(Boehringer-Ingelheim Economic Calculator)
Results: The reduction on gastro-intestinal antibiotic use in vaccinated group represents 56.4% compared to those animals that were not vaccinated(0.38^{a} vs 0.17^{b}€).
The average mortality was 19.87% lower in vaccinated group (1.56^{a} % vs 1.25^{b} %).
In addition FCR was 120 g less in vaccinated group(2.6^{a} vs 2.48^{b}).
The benefits cost ratio calculated with BECAL was 2.6:1€.
Conclusion: In this field experience, in a high health farm it was demonstrated that performance was improved due to the reduction on gastro-intestinal antibiotic ,mortality and mainly by reducing FCR with the vaccination with Enterisol Ileitis®.

Disclosure of Interest: None Declared

Keywords: Lawsonia intracellularis, oral vaccination

Poster Abstracts

Bacteriology and Bacterial Diseases
LAWSONIA

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Field experience with vaccinating gilts against ileitis via liquid feed

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Introduction: Vaccinating future gilts against Ileitis has become routine practice on many multiplying farms. When vaccination is carried around weaning it is typically applied via drench. While this is an effective and safe method, it is time laborious and might cause some stress to the animals. If epidemiology allows the easiest and least stressful way is oral application via drinking water in the nursery or at start of grow-finishing. This field experience describes Ileitis vaccination of gilts via liquid feed.
Materials and Methods: This field experience was made in a gilt multiplier farm with 350 breeding sows with a 3-week rhythm and weaning at 4 weeks of age. To control PHE caused by Lawsonia intracellularis in future gilts after selection Enterisol® Ileitis vaccination was introduced. First the vaccine was applied by drenching at 28 days of age. In addition gilts were treated with Tylosin-tartrate 100% (11 mg/kg body weight) for 3 days before and 4 days after selection (at 160 days of age). With this schedule PHE rate was reduced to < 0,5%. To reduce the workload the vaccine has been applied via liquid feed one day after placement into growing (about 85-90 days of age) from June 2013 onwards. It is visually checked if feed including vaccine is applied to all troughs (which is facilitated by colouring with Thiosulphate blue) and all pigs are eating properly.
Results: Vaccination via liquid feed was well tolerated by the pigs. Efficacy was demonstrated by the absence of typical clinical signs a PHE rate that continued to be < 0,5%, even when treatment with Tylosin was discontinued. In addition, the total complaint rate (including other diseases, infertility, malformations) for gilts delivered further decreased from 6,7 % in 2012 to 4,3% in 2014.
Conclusion: Drenching pigs is a practical way to vaccinate young pigs against Ileitis if pigs already get infected early in the nursery. However, drenching each individual pig is more time consuming than mass application methods. In addition, mass application via liquid feed avoids extra stress on the pigs, as they are not touched at all during the vaccination process. According to the epidemiology (seroconversion starting around 125 days of age) it can be even more effective to vaccinate via liquid feed at the age of 85 days to reduce PHE in the most stressfull period of selection at the age of 160 days. Due to late time of infection a period of at least 6 weeks between vaccination and seroconversion still is ensured. Taking clinical data and losses into account, application via liquid feed was at least as effective as drenching. Based on the positive field experience the farm continues to apply the oral live vaccine via liquid feed.

Disclosure of Interest: None Declared

Keywords: Lawsonia intracellularis, Vaccination via liquid feed

Bacteriology and Bacterial Diseases
LAWSONIA

PO-PF3-230

Effects of ENTERISOL ILEITIS against PHE in finishing pigs

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Introduction: PHE is an acute clinical form of proliferative enteritis that is often associated with young adult pigs 4 to 12 months of age and causes critical economic losses to the farmers because of mortality of the finishers(1). Even though antibiotics could be used to prevent and cure the PHE, it has a limited effect. The use of antibiotics pushes the disease to a later onset in the very late finisher stage. The present paper describes the reduction of PHE cases after Ileitis vaccine application.
Materials and Methods: This field case was recorded in a 1,500 head sow farm. It has 2 pig flows in different sites. Pigs are raised in the piglet producing site up to ten weeks of age and then transferred to the finisher site (continous flow). Pigs are slaughtered at about 24 to 27 weeks of age.
The herd is PRRS positive. PCV2 and Mycoplasma hyopneumoniae vaccination is done routinely in piglets at 3 weeks of age. (mixture of Ingelvac CircoFLEX and MycoFLEX, 2ml i.m.).
In 2013, due to a PHE outbreak, the mortality rate in finishers increased significantly. Peak mortality was observed at the age of 20 – 24 weeks. Beside acute cases some chronic cases of Ileitis were observed in the fatteners as well, mainly between 10 and 14 weeks of age. The farmer decided to use tylosin (1.5kg/feed ton) for 3.5 weeks in growing pigs between 8 and 10 weeks of age. As the high mortality persisted despite antibiotic treatment, Ileitis vaccination was implemented in October 2014. Pigs were vaccinated at 2 weeks of age via drench. The number of dead pigs was recorded prior and after the implementation of ileitis vaccination.
Results: The nursery mortality was slightly reduced from 100 heads/month before to 62 heads/month after implementation of vaccination.
Even though tylosin was added to the feed for growing pigs during 3.5 weeks continuously, the mortality in the finishers was still high with more than 150 pigs/month.
After vaccination, the number of dead pigs was reduced to 110 pigs/month with no signs of either chronic or acute form of ileitis (Figure 1).
Conclusion: This field case demonstrates that Ileitis vaccination is effective in protecting pigs against PHE. Mortality in late finishing was clearly reduced and no clinical signs associated with either the acute or chronic form of Ileitis were observed in the vaccinated pigs. The results described here are persistent with the findings of other researchers and other observations in the field (2, 3). The vaccination with ENTERISOL Ileitis protected the pigs well up to 24 to 27 weeks of age, i.e. up to 25 weeks after vaccination.

Disclosure of Interest: None Declared

Keywords: Enterisol ileitis, Lawsonia, PHE

Bacteriology and Bacterial Diseases
LAWSONIA

PO-PF3-093

Spreading of Lawsonia intracellularis in fattening pigs fed different diets

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Introduction: *Lawsonia intracellularis (L.i.)* is one of the economically most important pathogens in the swine production worldwide. This study tested the hypothesis that the composition of diets for fattening pigs has an impact on the excretion of *L.i.* in a natural infection model.
Materials and Methods: Fifty boars (~90 kg BW) from a SPF-farm were on site blood sampled and tested for antibodies against *L.i.* (percent inhibition: PI-values) and allotted to five feeding groups (with subgroups of five animals) during the last four weeks before slaughter. Groups were fed ad libitum either a finely ground pelleted diet (FP), a coarsely ground meal diet (CM), a usually ground meal diet either with 22 % cracked corn (CORN), 16.9 % dried whey (WHEY) or 30 % raw potato starch (RPS). Once a week the counts of *L.i.* were analysed in faeces by qPCR as well as the caecum content from slaughtered pigs. Samples of caecum wall were taken for histological analyses. Blood samples were analysed serologically for *L.i.* status. Statistical analysis was performed with SAS (Proc GLM).
Results: The animals in the different groups (FP/CM/CORN/WHEY/RPS) had a comparable serological status at the beginning of the feeding experiment (PI-values blocking ELISA: 31.0^{a}±12.9/ 30.5^{a}±16.9/25.5^{a}±13.1/29.0^{a}±14.7/32.9^{a}±14.6). Five weeks later, the test results of the serological examination were higher, even though the numerical difference between serological status at slaughter and the beginning of the trial did not differ between groups. In all subgroups shedding was detected in week 0 (lg GE *L.i.* /1 g faeces: 2.46^{a}±2.64/3.58^{a}±2.54/ 3.43^{a}±2.37/2.30^{a}±3.16/2.58^{a}±2.73). The number of *L.i.* microbes in faeces during the trial period did not differ between the groups (lg GE *L.i.* /1 g faeces - Ø week 1to4: 3.40^{a}±1.53/3.01^{a}±1.41/3.80^{a}±1.71/3.98^{a}±2.20/ 4.08^{a}±2.13). In animals fed the WHEY-diet, significantly lower amounts of *L.i.* were found in the caecum content (lg GE *L.i.* /1 g caecum content: 4.34^{ab}±3.83/5.46^{a}±3.03/5.16^{a}±3.63/ 1.57^{b}±3.32/5.82^{a}±3.31). In RPS-groups a significantly higher crypt depth, the significant lowest dry matter content in the faeces and an unfavourable performance on an overall high level existed (daily gains>1000g).
Conclusion: This study provides preliminary evidence that it cannot be excluded that dietary effects on the course of *L.i.* infection are possible. Further studies with experimental infection would be necessary to verify if certain sugars (lactose-group WHEY) or starch sources (group RPS) interfere with processes in a *L.i.* infection.
Parts of the project were supported by the Federal Ministry of Food and Agriculture, Germany.

Disclosure of Interest: None Declared

Keywords: diet, fattening pigs, Lawsonia

Bacteriology and Bacterial Diseases
LAWSONIA

PO-PF3-247

Boar taint in the context of dietary concepts and Lawsonia intracellularis infections in fattening pigs

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Introduction: Skatole and androstenone are the two main substances that are responsible for the so-called boar taint in pigs. Up to now studies are lacking concerning possible interactions between diet, *Lawsonia intracellularis (L.i.)* infection and skatole production.
Materials and Methods: Fifty SPF-finishing boars were tested for antibodies against *L.i.* and skatole levels and allotted to five feeding groups (n=2x5) four weeks before slaughter. Diets were either finely ground and pelleted (FP), ground coarsely (CM), usually ground with 22 % cracked corn (CORN), 16.9 % dried whey (WHEY) or 30 % raw potato starch (RPS). Weekly counts of *L.i.* were analysed in faeces by qPCR and assigned to shedding-classes (lg GE *L.i.*/1g: A=<5; 5≥B<6; 6≥C<7; 7≥D<8; E=≥8). For cross-group comparisons of skatole level in faeces, the values of the positive samples were corrected by the normal value of negative samples at identical feeding. At slaughter caecum content was analysed via qPCR. Neck fat and blood were analysed for skatole and androstenone levels, blood samples also serologically for *L.i.* (percent inhibition: PI-values). Differences in PI-values between start and slaughter were assigned to “PI-classes” (I: ΔPI<0; II: ΔPI 0-20; III: ΔPI>20-30; IV: ΔPI>30).
Results: At start, *L.i.* status (serological/qPCR) in groups (FP/CM/CORN/WHEY/RPS) was not different. Skatole levels in blood and in faeces did not differ within feeding groups concerning *L.i.* positive and negative pigs (qPCR). In the four week period only once differences in skatole levels (in µg/g DM) occurred between negative and positive faeces samples (FP:68.5vs64.3/CM:82.8^{a}vs.119^{b}/CORN:77.9vs.75.7/WHEY:82.9vs84.8/ RPS:30.5vs.66.3). Corrected skatole levels in positive faeces samples differed concerning shedding-class (A:13.0±38.6^{ab}/B:13.4±52.3^{b}/C: 2.98±45.7^{b}/D:-0.08±32.3^{b}/E:54.6±122^{a}). In faeces with > 8.5 lg GE *L.i.* /1g, there was a linear correlation to the skatole level (Pearson R=0.89; p=0.0028). Average of *L.i.* shedding was different between PI-classes (I:1.62±1.22^{b}/II:2.94±1.39^{ab}/III:3.39±1.38^{ab}/IV:4.61±1.77^{a}) during the trial. There was linear positive correlation between average of PI-values in PI-classes and lg GE *L.i.* /1g faeces during the trial (R=0.99; p=0.01) as well as average of androstenone in fat (R=0.96; p=0.04) at slaughter in these classes.
Conclusion: This study provides evidence that interactions between the skatole production in the intestinal tract may occur in case of a *L.i.* infection. Interactions between substrate availability, microflora and skatole production depending on diet require further investigations.
Parts of the project were supported by the Federal Ministry of Food and Agriculture, Germany.

Disclosure of Interest: None Declared

Keywords: boar taint, diet, Lawsonia

Poster Abstracts

Bacteriology and Bacterial Diseases
LAWSONIA

PO-PF3-198

Field observation: No PHE complaints after transport of replacement gilts and less antibiotics during 3 years of oral Lawsonia ileitis vaccination

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Introduction: Ileitis is caused by the bacterium *Lawsonia intracellularis* (Li), found all over the world in pig production systems. The general assumption is that for commercial swine operations it is very hard to become and stay Li free for a prolonged period of time. Porcine Hemorrhagic Enteritis (PHE) is a form of ileitis which is clinically recognized by acute hemorrhagic diarrhea and sudden death of replacement animals and finishing pigs close to market (Guedes 2004).
This paper describes a field observation in which cases of PHE in replacement gilts were controlled by oral Li vaccination.
Materials and Methods: A breeding herd of 330 sows with a conventional health status sells and delivers replacement gilts to 25 different multipliers with variable health statuses. In February 2012 cases of PHE in replacement gilts where reported after transport to multipliers. Oral Li vaccination (Enterisol Ileitis®) was started 4-8 weeks before transport, at 22 weeks of age, in all replacement gilts. After an outbreak of PHE early 2013 in gilts before the age of vaccination, an additional vaccination was implemented at the age of 12 weeks. The goal of Ileitis vaccination was reduction of PHE cases from 4 months of age until introduction in the sow herd at the multiplier farms.
Results: During 3 years of oral Li vaccination some outbreaks of PHE were reported in replacement gilts from 3 to 8 months of age, but never after transport to the multipliers. A peak in mortality of 4,2% in the first quarter of 2013 was observed in non-vaccinated gilts. Mortality from 3 to 8 months of age varied during the years, but in general was below 2% per quarter of a year.
Over the same period, less antibiotics were needed: Defined Daily Dosages (DDD) were reduced from 10.6 in 2011 to 2.4 in 2014.
Conclusion: No complaints about PHE after transport were received in any of the gilts orally vaccinated with ENTERISOL Ileitis against Li before onset of infection. In addition, less antibiotic treatments were needed during the raising of the gilts.The absence of PHE cases in transported gilts contributed to a sustainable client relationship for the breeding farm and her clients.

Disclosure of Interest: None Declared

Keywords: Lawsonia, PHE, Vaccination

Bacteriology and Bacterial Diseases
LAWSONIA

PO-PF3-133

Case study: Reduction of antibiotic use in a Dutch finishing farm after implementation of oral Ileitis

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Introduction: In the Netherlands antibiotic use in human health care and in the food producing animals is a concern for the Dutch society and the Dutch government. The use of antibiotics is controlled by law and the consequence is a strict monitoring in the diagnostics of diseases including laboratory tests to confirm disease and treatments. The message of the Dutch government is simple: use less antibiotics in animals. In this survey we demonstrate how in a finishing farm the implementation of oral vaccination against ileitis reduced antibiotic use.
Materials and Methods: A finishing farm, 1600 places, high standards of biosecurity, had a history of acute mortality in pigs close to slaughter, diagnosed as Porcine Hemorrhagic Enteritis (PHE).
The production results (feed conversion ratio and average daily gain) were at least equal to comparable finishing units without clinical ileitis. When mortality caused by PHE first started, antibiotics (tylosine) were used. This was with good effect; mortality dropped. The continuous use of antibiotics resulted in a high DDD (Defined Daily Dosage), well above the described goals used in the Netherlands. Oral ileitis vaccination (Enterisol Ileitis®) at one week after arrival was implemented to prevent the impact of mortality caused by PHE and to reduce antibiotics necessary to control the disease.
Results: The average production results in the year before vaccination compared to the results in 18 months of vaccinated pigs showed no change in Average Daily Gain, in Feeding Conversion Rate and in Mortality. At the same time the use of tylosine, antibiotic of choice to control ileitis, decreased from 30.3 KG per year to an average of 14 KG per year. After implementation of vaccination less compartments had to be treated with tylosine, which helped to the reduction of antibiotic use.
Conclusion: This survey shows a farm suffering from mortality caused by PHE. The use of antibiotics at this farm was, according to Dutch standards, too high. The effect of the antibiotics was sufficient, but offered no sustainable solution. Starting oral ileitis vaccination resulted in an average 46% reduction of tylosine (kg active matter) use. The mortality didn't change significantly. The achieved results have to be addressed to be a team effort: farmer, feed adviser and veterinarians each had an important role.
Our conclusion is that oral ileitis vaccination can be a helpful tool in reducing the use of antibiotics and is therefore a support in sustainable pig production.

Disclosure of Interest: None Declared

Keywords: antibiotic reduction, Lawsonia, Vaccination

Bacteriology and Bacterial Diseases
LAWSONIA

PO-PF3-238

PHE signs become first visible only weeks after initial Lawsonia intracellularis infection

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Introduction: Porcine Hemorrhagic Enteritis (PHE) is the most dramatic clinical form of ileitis caused by *Lawsonia intracellularis* (Li) and is characterized by acute hemorrhagic diarrhea and sudden death of replacement animals and finishing pigs close to market. Clinical PHE is mainly related to a batch infection from about 16 weeks of age. PHE has an on average low morbidity but a high mortality and over time usually occurs in outbreaks.
The objective of this study was to have an indication of the minimum time that passes between the initial Li infection and clinical signs of PHE and to elucidate whether the dramatic clinical signs are related to an acute infection with Li.
Materials and Methods: From May 2013 until August 2014 at the GD Animal Health a number of 48 ileitis suspected cases were sent in for necropsy. Gross lesions were used to confirm the clinical picture and Li infection was confirmed by histology and PCR testing. From the heart blood was taken and tested for Li-IgG antibodies (IFAT, Bioscreen). We used the IgG-positive test result as an indication for the minimal period that had passed between infection and death, having PHE signs.
Results: Based on gross lesions (anemia, presence of blood/ blood clots in the small and/ or large intestines) 22 of the 48 ileitis suspected cases were defined as PHE. Of these 18 PHE cases (82%) were serologically IgG positive at necropsy.
Conclusion: A previous study indicated that seropositivity in gilts did not prevent them from having clinical signs due to PHE signs a few weeks later, suggesting that PHE clinical signs were not a result from an acute primary infection. IgG-antibodies develop from 2 weeks after infection and peek around the end of the third week. After an experimental challenge (4-6 x 10^9) at 21 days post oral dosing 73% of the pigs were IFAT-positive and at 28 days 94%. Given the fact that 82% of the serum samples in this study were IgG-positive at the time of necropsy, we conclude that the initial Li infection took place at least 2 weeks before the animals died and, therefore, that clinical signs of PHE were the result of pathological changes in the course of the infection. The factors that contribute to these pathological changes which result in the clinically acute hemorrhagic diarrhea, still have to be elucidated.

Disclosure of Interest: None Declared

Keywords: Lawsonia, PHE

Bacteriology and Bacterial Diseases
LAWSONIA

PO-PF3-258

Using the acute phase protein Pig-MAP in diagnosing Ileitis

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Introduction: In a monitoring program finishing pigs at a farm were found positive for PRRS and Ileitis at the end of finishing. Pigs at the involved farm (7000 finishing pig places, Topigs 20 x Topie, boars & gilts) were vaccinated against PCV2 and Mycoplasma. Pleurisy and pneumonia were below the slaughterhouse average. Technical performance was lower than the Dutch average (ADG of 750 vs 796 gram/day). Pigs showed a growth depression in the grower period, but without clinical symptoms like respiratory problems and/or diarrhea which made it hard to select acute phase representatives for post mortem examination. To get more insight in the cause the use of acute phase proteins and antibody seroconversion during the growth depression phase were combined.
Materials and Methods: In total 5 consecutive week groups were selected for measuring the acute phase protein Pig-MAP using a commercial Elisa (PigCHAMP-Pro). The first week group was the group of pigs one week before showing the first signs of the growth depression, at 3 weeks of finishing. The subsequent week groups were at 4, 5 and 7 weeks of finishing, showing signs of growth depression. The last selected group was at 8 weeks of finishing, and were recovering. Of each week group, 20 pigs were randomly selected and 7 ml of blood was collected by jugular venapunture. After the analysis of Pig-MAP, 10 serum samples from week group 5 and 6, and 15 samples from week group 8 were selected for seroconversion against PRRS (Idex herdcheck Elisa) and ileitis (Bioscreen Illeitis Elisa).
Results: Pig-MAP was considered normal in the samples of week group 3 (0.59 mg/ml, sd 0.20 mg/ml). Subsequent week groups 4,5,7 and 8 showed a raise in Pig-MAP concentrations with means of 0.79 (SD 0.29), 0.81 (SD 0.29), 0.89 (SD 0.29) and 1.14 (SD 0.83) mg/ml respectively. All the selected serum samples for PRRS-analysis were positive with a mean S/P ratio of 2.11 (ranging from 1.09 until 2.69), indicating earlier infections before the observed growth depression. Seroconversion due to ileitis however, was in line with the observed growth depression. Positive samples were increasing from week group 5 until 8 (# negative/ # inconclusive/ # positive samples): group 5: (8/0/2); group 7: (5/2/3); group 8: (2/3/10). The raise of positive samples was in line with the raise of Pig-MAP concentrations per week group.
Conclusion: Using a combination of measuring growth depression, the use of the acute phase protein Pig-MAP and antibody seroconversion, made it possible to differentiate between PRRS and ileitis. Ileitis is the most plausible cause of the growth depression.

Disclosure of Interest: None Declared

Keywords: acute phase proteins, diagnosis, ileitis

Poster Abstracts

Bacteriology and Bacterial Diseases
LAWSONIA

PO-PF3-134

Field observation: Successful oral ileitis vaccination in liquid feed

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Introduction: Damage associated with ileitis due to infection with *Lawsonia intracellularis* (Li) can be controlled by oral Li vaccination. Application of the vaccine via drinking water is the preferred way of vaccination. On farms using liquid feeding systems the only way of mass vaccination is mixing it into the feed, as water uptake via the drinking water line is not reliable. This paper describes a field observation in which ileitis in finishers was controlled by oral Li vaccination in liquid feed.
Materials and Methods: A finishing site of 11,000 finishers, liquid feeding, all in – all out per room, starting weight 25-26 kg. The site had a history of ileitis related clinical signs and mortality. Serology confirmed Li infection. At the site liquid feed was prepared two times a day in a feed tank and pumped through pipelines to the pigs into storage feeders in two time periods of 3 hours per feeding.
Piglets, 2400 per batch, were orally Li vaccinated 4-6 days after arrival at the finishing site. No antibiotics on a batch level were allowed from arrival until 3 days after vaccination. For the oral Li vaccination, the feeding period before vaccination was decreased by 20% to make the pigs more hungry at the feeding with vaccine in it. For vaccination, first the liquid feed for the piglets that had to be vaccinated was prepared in the feed tank. Second sodium thiosulfate (55 grams per 500 liter of liquid feed) was added and mixed into the feed cocktail. Third the vaccine (Enterisol Ileitis®) was added into the feed cocktail and allowed to mix for several minutes before pumping it to the piglets that needed vaccination. The piglets consumed the feed with the vaccine at the storage feeders over a 5-6 hour time period.
Results: The production results that were obtained from the farm management system, the 6 months before and 6 months after vaccination were compared and showed an increase in Average Daily Gain (gram/ day; 757 versus 800), a lower Feeding Conversion rate (kg/ kg; 2.54 versus 2.43), a lower mortality (%; 3.2 versus 2) and a lower antibiotic use (Daily Defined Dosage; 10.1 versus 2.6)
Conclusion: After starting the oral ileitis vaccination in liquid feed, not only the production results increased, but also less antibiotics were needed. From six months before the vaccination started onwards, some feed ingredients were changed. This might also have contributed to the results before vaccination was effective.
The results at this finishing site strongly indicate that oral vaccination in liquid feed with Enterisol Ileitis® is working up to expectations, comparable to results that are achieved in farms that apply vaccine in the drinking water.

Disclosure of Interest: None Declared

Keywords: Lawsonia, liquid feed, Vaccination

Bacteriology and Bacterial Diseases
MYCOPLASMA

PO-PF3-229

Mycoplasmal pneumonia in pigs: effect of three different inoculation routes

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Introduction: One of the most distinguishing features of experimental *Mycoplasma hyopneumoniae* (*Mhp*) challenge systems is the inoculation route used. Therefore, the aim of this study was to assess the effect of different inoculation routes on mycoplasmal pneumonia (MP) outcome in pigs challenged with *Mhp* by studying pathological and immunological parameters.
Materials and Methods: Thirty *Mhp* seronegative piglets were assigned randomly into four groups. At 6 weeks of age, pigs were experimentally inoculated by endotracheal (ET; n=8), intranasal (IN; n=8) or aerosol (AE; n=8) routes. One group stayed uninfected (Control; n=6). Blood samples were collected before challenge and at necropsy to assess seroconversion. Laryngeal swabs were collected at -1, 7, 14, 21 and 28 days post-inoculation (dpi) in order to evaluate *Mhp* colonization by real time PCR (rt-PCR). At necropsy (28 dpi), lung lesions were scored and lung tissue was collected for *Mhp* DNA detection by rt-PCR. Broncho-alveolar lavage fluid (BALF) was collected from those three animals showing the most severe lung lesions within each group. These samples were used for *Mhp* DNA (rt-PCR), cytokines and specific *Mhp*-IgA antibody detection.
Results: MP was observed in all inoculated groups, but the ET group displayed significantly higher number of animals affected by MP, higher mean lung score, earlier seroconversion and upper respiratory tract *Mhp* colonization. In fact, while in the ET group *Mhp* DNA was detected from 7 dpi onwards, in both IN and AE groups, *Mhp* DNA was not detected until 21 dpi. Accordingly, the highest percentage of *Mhp* rt-PCR positive animals in lung tissue belonged to the ET group. All BALF samples from the three challenged groups were *Mhp* rt-PCR positive. However, higher levels of pro-inflammatory IL-1ß and IL-8 cytokines and *Mhp*-specific IgA antibodies in BALF were found in the ET group.
Conclusion: Under the conditions of the present study, the ET inoculation route was the most effective to induce MP in pigs experimentally challenged with *Mhp*. The inoculation route may have a certain impact on the inoculum infectious dose, which then is reflected in the *Mhp* colonization and in consequence, in the infection outcome. It cannot be ruled out that in the case of IN and AE routes, more than four weeks post-challenge would have increased the percentage of animals showing MP and its severity.

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Disclosure of Interest: None Declared

Keywords: Inoculation route, Mycoplasma hyopneumoniae, Mycoplasmal pneumonia

Bacteriology and Bacterial Diseases
MYCOPLASMA

PO-PF3-117

COMPARATIVE MYCOPLASMA HYOPNEUMONIAE SEROLOGICAL FOLLOW UP AFTER INTRADERMAL OR INTRAMUSCULAR VACCINATION OF GROWING PIGS IN THREE FRENCH FARMS

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Introduction: In many comparative field trials, *Mycoplasma hyopneumoniae* (Mhyo) vaccination by needle free, intradermal route has proven to be efficacious. This vaccination method appears less painful for the animals, and provides a well appreciated convenience for farmers. The aim of this study was to compare field serological data obtained from 3 groups of piglets intradermally (ID) or intramuscularly (IM) vaccinated, or non-vaccinated, in order to evaluate if serology is a good tool to monitor post-vaccination response particularly after ID application.
Materials and Methods: Sera originated from a comparative trial conducted on 2348 piglets from 7 farrowing batches in 3 farms in France. Six piglets were sampled for blood at 3, 10, 16, 22 weeks of age in each of 3 contemporary and comingled groups of animals, in each batch: 1) Negative control group (C), injected IM with Diluvac® placebo; 2) Vaccinated IM with Suvaxyn® M.Hyo mono; 3) Vaccinated ID with Porcilis® M Hyo ID Once using the IDAL® injector. The serological assays were performed by MSD R&D Service Lab (Boxmeer NL) using Mycoplasma Hyopneumoniae antibody TEST IDEXX®.
Results: A total of 126 animals, approximately 6 per treatment group in each of the 7 batches followed, were sampled at 3, 10, 16 and 22 weeks of age, resulting in 479 Mhyo ELISA results. At 3 weeks of age, the ELISA positive results (34%) was linked to maternal derived antibodies (MDA). In group C, none of the 10 and 16 week old piglets were positive following fade out of MDA, but 21% of pigs seroconverted by 22 weeks of age after a field Mhyo challenge.
Seven weeks after vaccination, 95% of previously Mhyo positive or suspect pigs in both vaccinated groups were seronegative, and only 7 % IM and 13 % ID pigs were Mhyo seropositive post-vaccination. At 22 weeks of age, a greater percent of IM (39%) and ID (55%) than C (21%) pigs became positive and average S/P ratios were also higher (IM-0,47 and ID-0,63) than in C group (average value 0,34).
Conclusion: Due to the late Mhyo field challenges found during this longitudinal study, it was clear that few IM or ID vaccinated pigs seroconverted. So we can conclude that serology is not a good tool to monitor the vaccination response following an intradermal application of a one dose Mhyo vaccine, as well as following an IM one dose Mhyo vaccination. However, the field infection did result in a booster effect, which was more obvious in the ID group that had higher S/P ratios.

Disclosure of Interest: None Declared

Keywords: intradermal vaccination, Mycoplasma, serology

Bacteriology and Bacterial Diseases
MYCOPLASMA

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Opportunities and limitations of Mycoplasma hyopneumoniae PCR testing in oral fluids to confirm involvement in respiratory disease.

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Introduction:
There are some doubts about the sensitivity of PCR testing in oral fluids (OF) for different bacterial respiratory pathogens including *Mycoplasma hyopneumoniae* (M. hyo). This study re-evaluated the potential use of M. hyo PCR tools in OF.
Materials and Methods:
Oral fluids were collected at 9 timepoints, each two weeks apart, from 5 to 21 weeks of age in six commercial all-in all-out wean-finish farms where pigs were M. hyo vaccinated at weaning. Six pens (16-120 pigs/pen) were sampled on each occasion in each farm with a rope:pig ratio of 1/25.
Samples were delivered on chill-packs to the laboratory within 20-26 hours of being collected. OF DNA was extracted using a high performance magnetic beads system (MagMax™ Pathogen RNA/DNA kit, Thermo Fisher Scientific®) and analysed by qPCR with a commercial kit (VetMAX™ M. hyopneumoniae Controls and VetMAX™ M. hyopneumoniae AB Design Reagents, Thermo Fisher Scientific®). Severity of respiratory disease was monitored clinically at sampling and by enzootic pneumonia (EP) -like lesion scoring at slaughter.
Results:
M. hyo was detected in OF in 4/6 tested farms, with positive results at least in 6 of the 9 timepoints. Detection was always negative in the first week after weaning (28-35 days old pigs), but positive results were found in 40-45 days old pigs. Pen-level prevalence was higher in older pigs; collections in >16 weeks old pigs were always positive in all the farms in which M. hyo was detected. Detection patterns were discontinuous at farm and pen level.
M. hyo detection in OF coincided with clinical respiratory signs. M. hyo PCR positive batches had higher EP-like lesion scores. On the contrary, few or no respiratory problems occurred on farms, and low prevalence or severity of slaughter lesions, where M. hyo was not detected.
Conclusion:
Based on the conditions employed in the present study, OF for surveillance of **M. hyo by PCR may be useful for confirmation of involvement in respiratory disease**. Certain correlations between M. hyo detection patterns (prevalence and Ct value), clinical respiratory problems and lesions in the respiratory tract were evident in this study.
The marked discontinuity of positive sampling timepoints on known positive farms highlighted the unsuitability of the present OF testing methodology to rule out M. hyo infection at a herd level. However, results in this study **highlight the correlation of M. hyo detection with clinical and pathological findings,** and its potential use in respiratory disease diagnostics in vaccinated herds.

Disclosure of Interest: J. Hernandez-Garcia Conflict with: Zoetis, Conflict with: University of Cambridge, N. Robben Conflict with: Thermo Fisher Scientific, D. Magnee Conflict with: Thermo Fisher Scientific, I. Dennis: None Declared, S. M. Kayes: None Declared, J. R. Thomson: None Declared, A. W. Tucker: None Declared

Keywords: Mycoplasma hyopneumoniae, Oral fluids, respiratory disease

Poster Abstracts

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Effect of pre-treatments on Mycoplasma hyopneumoniae detection in oral fluids

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Introduction: Oral fluids (OF) are an increasingly used sample type for monitoring swine pathogens on a pen basis. However, detection of *Mycoplasma hyopneumoniae (Mhp*) in OF has been indicated as having low sensitivity, especially during early stages of infection. Therefore, the objective of this study was to determine the effect of pre-treatments, such as thawing and holding temperature and sonication, in order to improve detection of *Mhp* in OF from experimentally infected pigs.
Materials and Methods: OF samples from 2 previous experiments were used for this study. A total of 12 OF were collected at 5, 9, and 21 days post-infection (DPI) and at 14 and 28 DPI from 2 different groups of *Mhp* experimentally infected pigs. OF from a *Mhp* negative herd were used as a negative control and a positive control was made by thawing negative OF, spiking them with a *Mhp* culture, and refreezing. Thawing temperature (4ºC and 25ºC for 22.5 hours), sonication (10 minutes), sonication temperature (4ºC and 25ºC) and sample holding temperature (4ºC and 25ºC for 2 hours) before DNA extraction were evaluated. Each OF was vortexed and aliquoted into 17 samples that varied based on pre-treatment conditions, totaling 222 samples. DNA extraction was performed using MagMAX® and *Mhp* qPCR using VetMAX®. The effect of pre-treatments on *Mhp* detection was determined through the comparison of Ct values within each sample.
Results: OF samples previously detected as *Mhp* negative resulted negative regardless of pre-treatment application. In the majority OF with high Ct values (≥35≤38), *Mhp* detection was numerically lower (higher Ct values) when samples were thawed at 25ºC, resulting in up to a 5.5 Ct value difference compared with thawing at 4ºC. The use of sonication, sonication temperature, and holding temperature did not seem to have an effect on *Mhp* detection in OF.
Conclusion: Under the conditions of this investigation, the use of pre-treatments did not seem to affect *Mhp* detection in OF. A numerical trend of higher Ct values was observed when OF were thawed at 25ºC. However, differences in this investigation were only numerical. Research focused on the effect of thawing temperature on *Mhp* detection in OF from naturally infected pigs is necessary to further evaluate this pre-treatment in samples with higher bacterial loads.

Disclosure of Interest: None Declared

Keywords: Detection, Mycoplasma hyopneumoniae, Oral fluids

Bacteriology and Bacterial Diseases
MYCOPLASMA

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Elimination of Mycoplasma hyopneumoniae from a 10,000 sow system using depopulation-repopulation, parity management and strategic medication

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Introduction: A 10,000 sow system consisting of five sow units with comingled weaned pig flow was endemically infected with *Mycoplasma hyopneumoniae* (Mhp). The goal of this project was to eliminate Mhp using a combination of depopulation-repopulation and parity management, with sow and piglet medication.
Materials and Methods: The five sow units were located within a 1.2 km radius circle. Sow units 1 and 2 were 150 m apart and consisted of 1,250 sows each. Sow units 3, 4 and 5 consisted of 2,500 sows each. Sow unit 2 had been depopulated and repopulated recently as part of a PED elimination project and showed no clinical signs of Mhp. Introduction of gilts into the sow farms was stopped in June 2014 (week 0). An off-site breeding project was done for depopulation-repopulation of sow unit 5, followed later by a second project for sow unit 3. Mhp exposed weaned P2+ females from sow unit 5 were used as replacement females for sow units 1, 3 and 4. Once sow unit 5 was restocked, unit 3 became the source of weaned P2+ replacement females for sow units 1 and 4, and finally unit 1 provided weaned P2+ replacements for unit 4. Pig flow from the repopulated units was segregated from the others and positive weaned pigs were placed as far as possible from the sow farms. Prior to introduction of negative gilts into sow units 1 and 4, lincomycin was included in the feed (220 g/T) for 30 days, and piglets were injected with tulathromycin (2 mg/kg) on day 1 and 10 of age, and weaned down to a maximum age of 14 days for two weeks before gilt introduction. Observation of clinical signs, Mhp-PCR on laryngeal swabs of targeted individuals and regular Mhp ELISA were integrated to monitor progress.
Results: Sow unit 2 remained negative throughout the project based on sentinel testing. Sow unit 5 repopulation was completed in week 27 and sow unit 3 in week 49. Both sow units and their pig flow continue to test negative by Mhp ELISA. Sow unit 1 began receiving negative gilts in week 56 and these have tested negative on Mhp ELISA. Sow unit 4 began receiving negative gilts in week 77. Non-vaccinated pigs in finisher sites housing the combined pig flow from sow units 1, 2 and 4 weaned from week 50 tested ELISA negative at 20 weeks post placement.
Conclusion: The off-site breeding project for two sow units allowed this Mhp elimination project to proceed with minimal impact on the flow of pigs from the system. The use of previously exposed (>240 days) mature females as replacements in the balance of the sow herds allowed the infection to die out of the population while maintaining sow inventories. The reduction in prevalence of Mhp reduced the infection pressure and risk of infecting the restocked negative sow units in close proximity.

Disclosure of Interest: T. Riek Conflict with: PIC, W. Hollis: None Declared, J. Kober: None Declared, J. P. Cano Conflict with: PIC

Keywords: Elimination, Mycoplasma hyopneumoniae

Bacteriology and Bacterial Diseases
MYCOPLASMA

PO-PF3-267

Practical experience with Porcilis® PCV M Hyo on a farm in the Czech Republi

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Introduction: *Mycoplasma hyopneumoniae* and PCV2 are both pathogens that can result in large economic losses in farms around the world. Vaccination against both pathogens has been an effective tool to prevent the disease and reduce such losses.
Recently, a first ready to use (RTU) PCV2 and Mhyo combination vaccine was registered in EU and was compared in a traditional Czech pig farm against the existing vaccination program.
Materials and Methods: The selected farm had 700 sows. Prior to the study, piglets were vaccinated against Mhyo at one week of age and against PCV2 at weaning (approx. 24 days of age). Piglets were also treated IM with antibiotics and iron during the first week after birth. Post- weaning, piglets moved to a wean to finisher unit in a different location. Sows are vaccinated against erysipelas, parvo, *E.coli* and PCV2.The farm remained free of acute disease outbreaks during the study .
The study included two groups of 1200 pigs each: Group 1) existing PCV2/Mhyo vaccination program; Group 2) Porcilis® PCV M Hyo (MSD AH) at weaning.
Four parameters were monitored during the study: 1) postvaccination reactions –observed immediately after vaccination; 2) performance of piglets in post-weaning period- animals were weighed before and after post-weaning period, and results were evaluated with farm software; 3) performance of pigs in fattening unit- animals were weighed before and after fattening period,and results were evaluated with farm and slaughterhouse software; 4) at slaughter, Mhyo lung lesions were scored with Bollo system (scale 0 – 4) and pleuritis prevalence was also recorded.
Results: There were no adverse events or negative impact on performance following vaccination. Vaccination with Porcilis® PCV M Hyo resulted in improved performance as demonstrated by 2.5 days less in the post-weaning unit and increased ADG by 15,25 g. In the finishing unit, FCR improved from 3,013 to 2,885, ADG increased by 31,5g and mortality loss reduced from 3,173% to 1,925%. Mhyo lung lesion scores were comparable between 2 vaccination protocols, but pleuritis prevalence in Porcilis® PCV M Hyo pigs was reduced by 4,5%.
Conclusion: In summary, Porcilis® PCV M Hyo improved performance of the pigs during the post-weaning and finishing phase in comparison with the existing vaccination program. In addition, administration of Porcilis® PCV M Hyo was convenient, reduced labor and improved animal welfare. Based on the experience and these results, the farmer decided to switch from existing vaccination program to Porcilis® PCV M Hyo.

Disclosure of Interest: None Declared

Keywords: None

Bacteriology and Bacterial Diseases
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Mycoplasma hyosynoviae colonization and lameness – a longitudinal investigation

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Introduction: *Mycoplasma hyosynoviae (Mhyos)* is a commensal of the oral cavity and nasal passages of pigs, and can persist in tonsils indefinitely. Conditions for systemic spread and disease development are not fully understood, yet clinical disease is characterized by a non-purulent polyarthritis in growing and finishing pigs that persists acutely for 3-10 days. It has been suggested that *Mhyos* may be present in the joints without observation of clinical illness, and pigs can recover with no further consequences. The epidemiology and colonization patterns of *Mhyos* are not fully described or mostly based on early diagnostic methods, such as culture. The bacterium is considered a re-emerging cause of lameness in the swine industry. The aim of this study was to characterize *Mhyos* colonization in a swine operation by employing molecular diagnostic techniques.
Materials and Methods: Sixty piglets were selected and individually identified from 29 sows at 3 days of age. Tonsil swabs were collected from sows and piglets at 1 and 3 weeks after farrowing. At weaning, piglets were distributed in 8 pens including 7 or 8 pigs along with other pigs of the same origin. Tonsil swabs and individual lameness scores (0-4) were collected from piglets at weeks 5, 7, 10, 13, 16, 19 and 22 of age. Oral fluids and pen-based lameness scores (0-4) were collected in all pens on the same weeks. Pigs that died or were euthanized had hind legs tested for *Mhyos* detection and histopathology evaluation. Pigs were considered positive for *Mhyos* colonization when genetic material was detected by real time PCR with Ct <37.
Results: Fifty five percent and 48.3% of sows were detected positive for *Mhyos* genetic material on tonsil swabs at weeks 1 and 3, while no genetic material was detected in piglets at the sow farm. After weaning, 3.8%, 5.8%, 48.1%, 67.3%, 71.2%, 60.8% and 60.8% of piglets were detected positive for *Mhyos* on tonsil swabs at 5, 7, 10, 13, 16, 19 and 22 weeks of age, respectively. On oral fluid samples, 25%, 75%, 63%, 97%, 100% and 100% of pens were detected positive at 5, 7, 10, 13, 16, 19 and 22 weeks of age, respectively. Mean individual lameness scores ranged from 0.06 to 0.23, and 0.01 to 0.08 for pen-based scores. Joint fluid samples were detected positive although no clinical presentation was observed.
Conclusion: Individual and pen-based lameness scores were low, and not associated with positive detection on tonsil swabs and oral fluid samples at any sampling point. Results of this study suggest that *Mhyos* is present in the herd even without clinical presentation. Thus, identifying the mechanisms of systemic spread need to be further investigated.

Disclosure of Interest: None Declared

Keywords: infectious arthritis , Lameness, welfare

Poster Abstracts

Bacteriology and Bacterial Diseases
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Diagnostic sensitivity of laryngeal swab samples for early Mycoplasma hyopneumoniae detection in vivo

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Introduction: A preeminent challenge faced by the swine practitioners is the identification of *in vivo* diagnostics combining accuracy and practicality to the point of strict correspondence to the real *Mycoplasma hyopneumoniae (Mhyop)* infection status of the herd. Laryngeal swabs (LS) are currently used for early detection of *Mhyop* and are suggested to have higher sensitivity in comparison to oral fluids and nasal swabs. However, their diagnostic sensitivity has not been reported in the literature. Therefore, the objective of this study was to evaluate the diagnostic sensitivity of LS samples by Bayesian estimation in order to support the validation of the technique as a standard diagnostic tool for *Mhyop* detection *in vivo*.
Materials and Methods: This investigation used results from a previous study where sixty gilts were dived into two groups, 21 gilts were experimentally infected with *Mhyop*, and 39 gilts were naturally exposed to experimentally infected gilts 28 days after inoculation. Gilts were allocated to one of six rooms, in a total of 10 gilts per room. LS were collected from experimentally inoculated gilts at 0, 14, 28, 42 and 56 days post inoculation (dpi), and from naturally exposed gilts at 0, 14 and 28 days post exposure (dpe). Bronchial swabs (BS) were collected from all gilts at 56 dpi and 28 dpe. Swabs were tested by *Mhyop* qPCR. Bayesian estimation was used to assess the diagnostic sensitivity of LS in comparison to BS, in the absence of a gold standard and assuming specificity less than 1. Three models with conditional dependent assumptions were performed. Model 1 comprised experimentally infected gilts data from LS collected at 14 dpi vs. BS collected at 56 dpi, and naturally exposed gilts data from LS collected at 14 dpe vs. BS collected at 28 dpe. Model 2 comprised experimentally infected gilts data from LS collected at 28 dpi vs. BS collected at 56 dpi, and naturally exposed gilts data from LS collected at 28 dpe vs. BS collected at 28 dpe. Model 3 was analyzed concurrently.
Results: The estimated mean sensitivity for model 1 was 82.4% for BS and 93.4% for LS. Model 2 resulted in 85.6% sensitivity for BS and 87.7% for LS. Lastly, model 3 resulted in 84.3% sensitivity for BS and 93.8% for LSS. The credible interval of all three models was included in the same range. The Deviance Information Criteria estimated was 28.3, 36.1 and 47.5 for Models 1, 2 and 3, respectively.
Conclusion: LS at 14 dpi or dpe were comparable to BS at 56 dpi and 28 dpe for early *Mhyop* detection in infected gilts in experimental settings. Among the *in vivo* diagnostics tools currently in use by practitioners, LS is proposed to be an accurate alternative to BS for early *Mhyop* identification in live pigs.

Disclosure of Interest: None Declared

Keywords: Bayesian estimation , laryngeal swab, Molecular diagnostics

Bacteriology and Bacterial Diseases
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Efficacy of one shot vaccination against an experimental infection with two Mycoplasma hyopneumoniae strains

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Introduction: *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) is the causative agent of enzootic pneumonia in pigs. In commercial pig herds, pigs may be infected with different *M. hyopneumoniae* strains. However, most challenge infection studies have used only one strain of *M. hyopneumoniae* to inoculate the pigs. The objective of the present study was to assess the efficacy of vaccination against experimental infection with two *M. hyopneumoniae* strains in weaned piglets.
Materials and Methods: Forty-five 33-day old (D0) *M. hyopneumoniae* free piglets were assigned to 3 groups : 1) negative control group (NCG; n=5), not inoculated and not vaccinated, 2) positive control group (PCG; n=20), inoculated but not vaccinated, and 3) vaccination group (VG; n=20), inoculated and vaccinated (D1) with Hyogen® (Ceva). At D23 VG and PCG were inoculated with 7ml 10^7CCU of highly virulent strain F7 and at D24 with low virulent strain F1. The pigs were euthanized at D53. Parameters of interest were: respiratory disease score (RDS), macroscopic lung lesions (MLL), concentration of *M. hyopneumoniae* DNA in BALf detected by qPCR (D53), IL1, IL6, TNFα, IgG and IgA OD values in BALf (D39, D53), histopathology and percentage of air in the lung tissue (D53).
Results: Only parameters with statistically significant differences ($P<0.05$) between groups are mentioned in following order with different superscripts per parameter: NCG, PCG and VG. The RDS was 0^a; 0.68^b and 0.32^c. The MLL was 0^a, 7.56^b and 0.68^a. The qPCR (D53) results were 0.40^a, 3.99^b and 1.78^a log copies. The results for the immunological parameters (D39) and (D53) for IL1 (D53)17.6^a, 1283.4^b;53.0^a pg/ml. The results for IL6 were: 130.3^{ab}, 242.2^a, 164.6^b and 148.1^a, 493.3^b, $259,8^a$ pg/ml. The IgG and IgA (D53) results were respectively 0.670^a, 2.540^b, 2.700^b and 0.866^a, 2.786^b,2.762^b. The results of the histopathology and air analysis was: 1.26^a, 3.39^b,2.12^c respectively 47.7^a, 34.5^b and 45.2^a percentage of air.
Conclusion: Hyogen® was efficacious against a double strain *M. hyopneumoniae* challenge infection in pigs. Vaccination significantly reduced clinical signs, inflammatory cytokine reponses and microscopic lung lesions against experimental infection with two different *M. hyopneumoniae* strains.

Disclosure of Interest: None Declared

Keywords: Mycoplasma hyopneumoniae, one shot vaccination, two strain challenge

Bacteriology and Bacterial Diseases
MYCOPLASMA

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Mycoplasma hyopneumoniae microbial load and histological lesion reduction in piglets vaccinated with MycoFLEX®

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Introduction: Pig vaccination against *Mycoplasma hyopneumonie (Mhp)* is the standard control tool for Enzootic Pneumonia. The economic benefits of vaccination have been reported in several studies and vaccination protocols are most effective when used simultaneously to other management procedures. The advantages of *Mhp* vaccination are: a) improvement of daily weight gain; b) improvement of feed conversion ratio; and c) reduction of clinical signs and lung lesions. It has been proposed that this effect is due to the ability to reduce the number of microorganisms in the respiratory tract and the ability to reduce damage on the lung tissue. The objective of the study was to evaluate the ability of MycoFLEX® in pigs of 3 weeks of age on the reduction of microbial load and microscopic lesions after challenged with *Mhp.*
Materials and Methods: At 3 weeks of age 180 piglets were randomly allocated in two treatment groups (T1 non vaccinated and T2 vaccinated), the treatment group size of 90 pigs per group was derived from a calculation to achieve a power of 0.80 at an alpha level of 0.05. On day 0 (21 days of age) of the study, T2 piglets were vaccinated; on day 28 and 29 of the study piglets from both groups where challenged intratracheally with *Mhp* strain 232. On study day 56, pigs were euthanized and formalin fixed lung tissues were submitted for histopathological scoring. In addition, bronchial swabbing samples and Bronchial Alveolar Lavage (BAL) samples were taken for *Mhp* qPCR and lung lesions were scored according to PigMON methodology.
Results: Statistical analysis revealed that vaccinated group had a significant reduction ($P<0.01$) of 30 % on *Mhp* qPCR results in both BAL and Bronchial Swabs as compared with non-vaccinated. Microscopic lung scoring was 2.17 for T1 and 1.84 for T2 ($P<0.05$) and PigMON lung scoring was 13.00 forT1 and 8.8 for T2 ($P<0.05$). No significant difference was observed on *Mhp* load between BAL and Bronchial Swabs samples in either vaccinated or non-vaccinated piglets ($P>0.05$).
Conclusion: Under the conditions of this study vaccinated piglets had a reduction in colonization as compared to non-vaccinates, documenting that qPCR on BAL or bronchial swabs samples can be a suitable method for microbial load assessment as part of *Mhp* vaccine efficacy evaluations. This effect leads on the significant improvement in histopathologic and lung lesion scoring compared to non-vaccinated pigs, documenting a clear modification of the *Mhp* pathogenesis. This study confirms the importance of pig vaccination to minimize the pathogenic effect in pigs exposed to *Mhp* and shows that Ingelvac MycoFLEX® is effective in aiding in the reduction of Enzootic Pneumonia.

Disclosure of Interest: None Declared

Keywords: Lesions, Mhyo, Microbial load

Bacteriology and Bacterial Diseases
MYCOPLASMA

PO-PF3-212

Detection of Mycoplasma hyosynoviae and Mycoplasma hyorhinis during a herd closure program

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Introduction: *M. hyosynoviae* (MHS) and *M. hyorhinis* (MHR) cause arthritis in post-weaning pigs. There is a lack of information regarding the duration of shedding and changes in the dynamics of infection of MHR and MHS when herd closure is applied. The objective of this study was to determine whether the implementation of herd closure can have an effect on the prevalence of MHS and MHR infection
Materials and Methods: The study was conducted at a 1,150 farrow-to-finish sow farm with previous history of MHS and MHR associated lameness in grow-finish pigs. The herd was undergoing a *M. hyopneumoniae* (MHP) elimination project, based on herd closure and strategic medication. A total of 462 oropharyngeal swabs (OS) and oral fluid (OF) samples were collected at five different times and tested by PCR. At baseline, OS were collected from 60 sows, 60 gilts, 60 weaning piglets, 30 70 d and 130 d pigs. Ninety OF were collected from sows, gilts, pre- and post-weaning pigs at 93, 145, and 185 days post herd closure. Finally, 60 OS and 12 OF samples from sows (p1) and 60 OS from weaning piglets were collected at 221 days post herd closure.
Results: At baseline, MHS was detected in OS in 68% of sows, 37% of gilts and 7% of piglets. Similarly, 22% of the sows, 32% of gilts and 8% of piglets were MHR PCR-positive. In post-weaning, 13% of 70 d and 16% of 130 d pigs were MHS PCR-positive. In contrast, 97% of 70 d and 43% of 130 d pigs were MHR PCR-positive. At 93 days post herd closure, MHS and MHR were detected in OF in all groups. MHR was no longer detected in OF at days 145 and 185 post herd closure, while MHS was detected in >73%. At 221 days post herd closure, MHR was detected in OS in 5% of p1 sows and in 8.3% of OF from sows. MHS was detected in 3.3% of piglets and 10% of sows in OS and 33.3% of OF from sows.
Conclusion: The detection of MHS and MHR in OS from weaning piglets suggests that pigs may become infected from their dams. Low sample size, antibiotic treatment and intrinsic differences between sample groups are some potential explanations as to why MHR was not detected in OF at 145 and 185 days post herd closure. A numerical decrease was observed between the baseline and final sampling, suggesting a possible effect of herd closure or recent antibiotic treatment over bacterial shedding. Detection of MHS and MHR before the herd re-opened indicates that the duration of shedding for both could potentially be longer than 222 days. However, success of herd closure depends on whole herd exposure prior to the start of herd closure. Not all animals were exposed to MHS and MHR before herd closure, as seen in the baseline results. Further research is needed to determine the duration of shedding for MHS and MHR.

Disclosure of Interest: None Declared

Keywords: Herd closure, Mycoplasma hyorhinis, Mycoplasma hyosynoviae

Poster Abstracts

Bacteriology and Bacterial Diseases
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Coughing Index in late fattening and Mycoplasma hyopneumoniae lung lesion scores in fatteners with- and without a combined PCV/M. hyo vaccination

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Introduction: *Mycoplasma hyopneumoniae* remains one of the most important pathogens in global swine production systems and is also widespread in the German pig population. Although it is known that *M. hyo* causes high economic losses, some farmers refuse to vaccinate against this pathogen in order to avoid cost and workload. Mild clinical signs are tolerated as long as no obvious clinical and economic disadvantages occur and no expensive treatment is needed. The objective of this observational study was to compare the clinical impact of two farm strategies: one without *M. hyo* vaccination, the other one with a new PCV / *M. hyo* combined vaccine.
Materials and Methods: The investigation took place in a semi-closed herd with appr. 1000 Sows, 7000 piglets and 8000 fatteners. Fatteners were housed in a fully slatted barn and had 24h free access to feed and water. Typical *M. hyo* clinical signs like dry coughing had been observed in the late fattening period and a slaughter lung check (SLC) displayed typical apical lesions. *M. hyo* infection was confirmed in lung samples by PCR and immunohistochemistry and in serological samples by ELISA (Idexx). Standard piglet vaccination was Ingelvac CircoFlex® (14 days of age), but no *M. hyo* vaccination. In order to reduce the clinical respiratory symptoms, vaccination with Porcilis® PCV M Hyo (PCVM) was initiated (21 days of age). Coughing index (n coughs/10 min/n pigs) was recorded in the late fattening period every 4-6 weeks between 10 am and 2 pm after encouraging pigs to get up. SLC was performed according to Madec and Kobisch in the same frequency. Statistics were calculated with SPSS Wilcoxon-Mann-Whitney-U Test, two sided, 95% CI.
Results: After the introduction of PCVM vaccination, coughing index was clearly reduced in the late fattening period (0.07 before vs. 0.03 after start of *M. hyo* vaccination; n=1475 vs. n=4589; p <0.001). Based on SLC, completely healthy lungs were significantly increased (Madec score = 0) following PCVM vaccination (2.34% before vs. 29.69% after vaccination; n=216 vs. n=549; p <0.0001). The average Madec score was reduced significantly in PCVM vaccinated pigs, PCVM= 2.7 and non-*M. hyo*=8.5. The percentage of lungs with pleurisy was reduced from 8.76 before to 4.57 after vaccination (p = 0.0274).
Conclusion: Porcilis® PCV M Hyo vaccination reduced coughing and improved respiratory signs under the conditions of this farm. These clinical observations were supported by healthier lungs at slaughter, expressed by lower Madec scores, more completely healthy lungs and a lower percentage of lungs with pleurisy

Disclosure of Interest: None Declared

Keywords: Mycoplasma hyopneumoniae, Coughing index, Slaughterhouse check

Bacteriology and Bacterial Diseases
MYCOPLASMA

PO-PF3-263

Efficacy of Mycoplasma hyopneumoniae vaccination at or shortly before weaning under field conditions

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Introduction: The aim of this study was to investigate the efficacy of a one-shot vaccination applied either at weaning or 3 days before weaning in a herd infected with *M. hyopneumoniae* (*Mhyo*) and other respiratory pathogens.
Materials and Methods: At 14 days of age, 828 piglets were randomly divided into 3 groups of 276 piglets each: 2 groups were vaccinated with Ingelvac MycoFLEX® either 3 days before weaning (V1) or at weaning (21 days of age, V2); a third group was left non-vaccinated (NV). After the nursery period 304 pigs were allocated to fattening unit 1 (F1) and 500 pigs to fattening unit 2 (F2). Pigs from the 3 treatment groups were equally allocated across F1 and F2. Diagnostic investigation included necropsies of dead pigs, serology against *Mhyo* and other respiratory pathogens, and quantitative real-time (qPCR) for the detection of *Mhyo* DNA on tracheobronchial swabs at 10, 14 and 18 weeks of age (same 20 pigs/group). Average daily weight gain (ADG), respiratory disease score (RDS), mortality rates and pneumonia lesions at slaughter (27 weeks of age) were assessed.
Results: Necropsies and serology showed the presence of secondary bacteria (*Trueperella pyogenes*, *Pasteurella multocida* and *Streptococcus sui*s), and also chronic infection with porcine reproductive and respiratory syndrome virus and porcine circovirus type 2. Overall, 50/60 pigs were seropositive for *Mhyo* one week prior to slaughter and 18/60 of those pigs were positive by qPCR at 18 weeks of age. Additionally, in F1 an outbreak of swine influenza H3N2 was confirmed at 20 weeks of age. Statistically significant differences were obtained in F2 where group V1 had higher weight gains and ADG compared to groups V2 and NV (P<0.05). When taking into account both fattening units, the ADG for the entire period in groups V1, V2 and NV were 610, 600 and 593 g, respectively (P=0.164) and group V1 was the only group where coughing severity did not increase significantly between the initial stage (10 to 20 weeks of age) and the end of the fattening period (20 to 26 weeks of age) (P>0.05). Regarding the mortality rates and lung lesions at slaughter, and also the weight gains and ADG in F1, differences between group V1 and groups V2 and NV were statistically non-significant.
Conclusion: In conclusion, vaccination against *Mhyo* ahead of weaning provided significantly better performance in F2. When taking both fattening units, performance of both vaccinated groups was numerically better but did not reach statistical significance. It can be assumed that especially the influenza outbreak in F1 and the fact that both F1 and F2 were facing mixed respiratory infections have influenced the performance of both vaccinated groups across all measured parameters.

Disclosure of Interest: None Declared

Keywords: Mycoplasma hyopneumoniae, Vaccination, Weaning

Bacteriology and Bacterial Diseases
MYCOPLASMA

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Decision tree to confirm herd-negativity for Mycoplasma hyopneumoniae using serology and clinical signs, followed by PCR and IHC

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Introduction: Pig populations, especially multiplier herds free of *Mycoplasma hyopneumoniae* (Mhp) are usually monitored to confirm their Mhp-negative status. Serology is the diagnostic tool used to screen herds. However, specificity is not 100% and thus false positive results are expected, which need to be further investigated to rule out potential recent infection as opposed to "false" alarm.
The purpose of this study was to build a decision tree for Mhp monitoring using serology results, clinical signs and PCR from nasal or lung samples.
Materials and Methods: *Herds and samples.* 30 blood samples were taken in a monthly interval for 6 consecutive months from 7 Mhp-free herds (5 sow farms, 2 boar studs). At the 7th month, sample size was increased to 60 blood samples. Herds were stoked from Mhp-free source and considered Mhp-free based on routine serology, PCRs, clinical signs and slaughter checks.
Also, 30 blood samples were taken from 7 Mhp-positive herds.
Diagnostics. Sera were tested with IDEXX ELISA. Herds were monthly assessed with coughing score and had ≥ 60 lungs evaluated for Mhp-like lesions in monthly slaughter checks. Donor pigs from ELISA-positive samples (in Mhp-negative herds) were nasal swabbed for PCR and had lung submitted for PCR from bronchial swab and histopathology.
Results: Mh-negative and Mh-positive herds had ELISA S/P mean (25th, 75th percentile) of 0.046 (0.012, 0.105) and 0.647 (0.207, 1.157) respectively. The proportion (and range) of ELISA positive results (i.e. S/P value ≥ 0.400) for Mh-negative and Mh-positive herds was 1.76% (0.0% to 8.4%) and 39.5% (14.03% to 64.97%) respectively.
It was not surprising that positive ELISA results were found in Mhp-negative herds. Assuming the test specificity of 98.6%, the likelihood of resulting in at least one false positive result is of 35% when 30 samples are evaluated (Rovira, 2011).
All nasal and lung PCRs from Mh-negative herds resulted negative, confirming false-positive results of serology. Based on results of this study, we propose a decision tree for Mhp monitoring of Mhp-negative pig populations based on S/P ratios, clinical signs and proportion of positive results. Briefly, in absence of dry coughing (and no use of anti-Mh drugs), on ELISA testing of ≥ 30 pigs with <10% prevalence of positive results and S/P ratios < 0.80 are usually Mhp-negative flows. When prevalence is greater than 10% or S/P ratios > 0.80 further PCR testing is required to confirm Mhp negative status.
Conclusion: Using the proposed decision tree allows veterinarians to take informed, science-based decision, saving time and automating decision-making of Mhp-negative flows.

Disclosure of Interest: None Declared

Keywords: Diagnostics, monitoring, Mycoplasma hyopneumoniae

Bacteriology and Bacterial Diseases
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Evaluation of a nested PCR to identify a repeat motif p97 in order to detect Mycoplasma hyopneumoniae genetic diversity

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Introduction: *Mycoplasma hyopneumoniae* is the primary agent involved in porcine enzootic pneumonia (EP). Different genotypes of M. hyopneumoniae have been described using Multiple Locus Variable-number Tandem Repeat Analysis (MLVA). In our experience it is difficult to perform a MLVA from nasal swab samples due to the sensitivity of some PCRs. In this regard, we have reported the increasing of the sensitivity of p146 locus (Tamiozzo 2011, 2013) developing a nested PCR. Since a recent study (Dos Santos 2015) showed a large number of M. hyopneumoniae types combining p146 and p97 loci and the possibility to type M. hyopneumoniae from minimally invasive samples, such as nasal swabs, without killing animals or performing invasive sampling, the objective of this study was to evaluate a nested PCR for M. hyopneumoniae typing.
Materials and Methods: A total of 56 M. hyopneumoniae PCR positive (16SrRNA) DNA samples (from nasal swabs, tracheo-bronchial lavages and Mycoplasma hyopneumoniae bacterins) were analyzed, amplifying p97 loci, by 3 PCRs: 1) PCR reported by de Castro (2006), 2) PCR reported by Vranckx (2011) and 3) a nested PCR format using for the first round of amplification, primers and conditions reported by de Castro et al., 2006 and for the second round, primers and conditions reported by Vranckx et al., 2011. Proportions of positives from each single PCR format were compared vs nested PCR format (chi ^{2} test, Epidat).
Results: Fifteen out of 56 (26.8%) samples were positives to the PCR reported by de Castro et al. (2006) whereas 18/56 (32.1%) samples were positives to the PCR reported by Vranckx 2011. Using the nested PCR format, there was 50% of positives. There was statistic significant difference (p=0.019) between the PCR described by de Castro vs the nested PCR format.
Conclusion: The use of the nested format allowed detecting a greater number of positive samples surely due to the increase of the sensitivity. While Vranckx et al. (2011) only worked with bronchoalveolar lavages and tracheal swabs, de Castro et al. (2006) suggested that the PCR assays could have sufficient sensitivity for M. hyopneumoniae typing from clinical samples, we think, a characterization by MLVA is not always possible using conventional PCR (Tamiozzo 2013, 2015), even when working with tracheal and/or bronquial specimens. Kuhnert et al. (2011) noticed that successful genotyping was dependent on a sufficiently high concentration of M. hyopneumoniae DNA in lung samples. We are aware that study of other genomic regions could have been useful to type M. hyopneumoniae, but others specific nPCRs have yet to be developed.

Disclosure of Interest: None Declared

Keywords: MLVA, Mycoplasma hyopneumoniae, p97

Poster Abstracts

Bacteriology and Bacterial Diseases
MYCOPLASMA

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In vitro antibiotic susceptibility of field isolates of Mycoplasma hyopneumoniae and Mycoplasma hyorhinis from South Korea

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Introduction: Swine mycoplasma *Mycoplasma hyopneumoniae* (Mhp) and *Mycoplasma hyorhinis* (Mhr) are respiratory pathogens in pigs. They are associated with enzootic pneumonia (EP) and porcine respiratory disease complex (PRDC) causing huge loses to the porcine industry. Currently commercial vaccines are available only against Mhp; however, their protection ability is not complete. Therefore, use of antimicrobials often becomes necessary to limit the disease in the event of outbreak or as an additional measure to prevent mycoplasma disease. However, there is no recent information on antibiotic susceptibility of these mycoplasmas from South Korea. In the present study, minimum inhibitory concentration (MICs) of South Korean Mhp and Mhr isolates to tylvalosin and other commonly used antibiotics (tiamulin, lincomycin, tilmicosin and chlortetracycline) was determined.
Materials and Methods: Mhp and Mhr field isolates (twelve each), obtained from enzootic pneumonia (EP)-like lung lesions during 2009-2011 from South Korea. Each of these isolates was passaged 6 times before using in the antimicrobial assay. Both Mycoplasma species were propagated in Friis broth and used at final concentration of approximately 1×10^5 cells/mL. MICs were determined using the broth microdilution method. Readings were taken after incubation of 7 days at 37°C, and the lowest concentration of antimicrobial inhibiting any detectable color change of the medium was defined as MIC of the drug.
Results: Tylvalosin showed highest activity against both Mhp and Mhr field isolates with MIC_{90} value of 0.06 µg/mL and 0.12 µg/mL, respectively. Tiamulin, lincomycin and tilmicosin also showed low MICs with MIC_{90}s of 0.12 µg/mL, 0.5 µg/mL and 4 µg/mL for Mhp isolates and MIC_{90}s of 0.25 µg/mL, 1 µg/mL and 4 µg/mL for Mhr isolates. The MIC_{90} value of chlortetracycline was 64 µg/mL for both Mhp and Mhr field isolates.
Conclusion: The results indicate the antibiotic tylvalosin has highest efficiency against Mhp and Mhr field isolates. To conclude, *in vitro* susceptibility must be taken into consideration while treating these swine mycoplasma in South Korea and will help for a choice among several antibiotics.

Disclosure of Interest: None Declared

Keywords: Minimum Inhibitory Concentration, Mycoplasma hyopneumoniae, Mycoplasma hyorhinis

Bacteriology and Bacterial Diseases
MYCOPLASMA

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Genetic variability of Mycoplasma hyorhinis in German and Swiss pig farms

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Introduction: *Mycoplasma hyorhinis* is a common inhabitant of the upper respiratory tract and tonsils of pigs. Its role as a potential respiratory pathogen remains controversial. Information on the population structure of *M. hyorhinis* might help to get a clearer picture on specific strains in the pig population involved in clinical disorders. Using multilocus sequence typing (MLST) we therefore genetically characterized *M. hyorhinis* isolates from Swiss and German pig herds previously included in a transnational cross sectional study determining the prevalence of *M. hyorhinis*.
Materials and Methods: A fresh culture of 60 *M. hyorhinis* isolates from stocks kept at -80°C was prepared and an aliquot was lysed at 96° C for 15 minutes and then submitted to PCR. All primers for the six target genes used in the MLST scheme of Tocqueville *et al.* (J.Clin.Microbiol. 2014) were newly designed to allow amplification and sequencing with a single protocol. The resulting sequences of the target genes *adk, dnaA, gltX, gmk, gyrB,* and *rpoB* contained the corresponding sequence parts used for allele definition on the PubMLST database (pubmlst.org). Edited sequences were entered to Bionumerics® and sequence types (ST) defined using the MLST plugin. New alleles and allele combinations were submitted to the PubMLST database.
Results: A total of 25 ST were observed with the 60 strains, 24 of them were new types. Only one ST was previously observed with a French isolate. Generally identical genotypes were observed within most farms. The same genotype was also observed in three different Swiss farms. On the other side different genotypes were found within 3 German farms and even within single animals from German farms.
Conclusion: The study revealed that MLST based on the six housekeeping genes is a very useful tool to analyze *M. hyorhinis* genetic variation and population structure. Data obtained shows a high variability of the *Mycoplasma hyorhinis* strains with, however, some limited clonality. The high diversity of both Swiss and German strains indicates that recombination of the *M. hyorhinis* genome is common and similar to what is observed for *M. hyopneumoniae*. Similar to this pathogen the population structure of *M. hyorhinis* also shows some limited clonality with predominant genotypes within a single herd and different ones between herds. This is more pronounced with Swiss strains where genotypes seem somewhat more conserved than strains in German farms. The similar population structure of *M. hyopneumoniae* and *M. hyorhinis* could indicate a kind of co-selection of the two species.

Disclosure of Interest: None Declared

Keywords: clonality, MLST, Mycoplasma hyorhinis

Bacteriology and Bacterial Diseases
MYCOPLASMA

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Ante-mortem vs. post-mortem sampling procedure comparison for the detection of Mycoplasma hyopneumoniae by PCR and the influence of pooling on results

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Introduction: Ante-mortem detection of *Mycoplasma hyopnuemoniae (Mhp)* can be difficult during the mid stages of an infection and in vaccinated populations. Commonly used methods to determine herd status such as nasal swabs, oral fluids, and oropharyngeal swabs, have shown low sensitivity and antibody testing is inconclusive in vaccinated populations. A more sensitive ante-mortem procedure is needed. The objective of this study was to compare ante-mortem tracheo-bronchial (TB) catheter and laryngeal sampling (LS) procedures to post-mortem bronchial swabbing (BS) (the reference standard) by individual and pooled samples for detection of Mhp by polymerase chain reaction (PCR).
Materials and Methods: Three groups of 35 non select gilts approximately 180 days of age were sampled from 3 separate gilt development units with known Mhp exposure beginning at 60 days of age. Each gilt was tagged to be identified at marketing; TB, LS, and serum samples were collected from each gilt. TB samples were collected using a 60 cm catheter passed orally. Immediately after, LS were taken with a smooth, long handled spoon and transposed onto a swab. The next day gilts were followed to the harvest facility where post-mortem bronchial swabs were collected individually. All samples taken were frozen at -80°C. Following all collections, samples were simultaneously submitted for Mhp PCR testing. Blood samples were tested for Mhp antibodies by IDEXX ELISA. Pools of 2, 3 and 5 samples were also tested for Mhp by PCR in submision order. A stochastic model was used to estimate the case detection rate with differing prevalence and sample size for LS.
Results: TB and LS had similar individual sensitivity (42.4% and 47.8%). BS had a significantly lower Mhp PCR cycle quantity values (29.2) when compared to TB (32.1) and LS (33.2). TB and LS had similar sensitivity results for pools of 2:1 (41.6% and 43.3%), 3:1 (64.1% and 53.8%), and 5:1 (66.7% and 70.8%) and were less sensitive than BS pools of 2:1 (91.1%), 3:1 (93.5%), and 5:1 (100%). Serum samples were 95% percent ELISA positive.
Conclusion: As expected, TB and LS were less sensitive compared to BS; however, these are currently the most sensitive ante-mortem sampling procedures available. As ante-mortem samples, TB and LS allow for an increased sample size compared to post-mortem BS and an alternative to ELISAs for vaccinated animals. Utilizing pooling and increasing sample size allows for a higher herd detection rate while pursuing the most economical approach. LS were easier to perform and train in the field than TB and would be recommended by the authors for Mhp detection in the early and mid stages of an infection and in vaccinated populations.

Disclosure of Interest: None Declared

Keywords: Mhyo, Pooling, Sampling

Bacteriology and Bacterial Diseases
MYCOPLASMA

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Mexican swine industry survey on Mycoplasma hyopneumoniae gilts aclimatation

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Introduction: Replacement gilt introduction as well as their management has to be considered as a risk factor for admittance and perpetuation of diseases in the farm. The objective of this study was to understand the replacement gilt acclimation process for Mycoplasma hyopneumoniae (M. hyo) in swine farms in Mexico.
Materials and Methods: Boehringer Ingelheim developed a survey composed of 14 questions with the objective of identifying which gilt acclimation methods for *M. hyo* are being used nowadays. The survey was completed by 51 veterinarians and producers *M. hyopneumoniae* positive sow farms, which were interested in the control of the pathogen. In total they represented 397,553 sows from different states in Mexico including Sonora, Jalisco, Michoacan, Guanajuato, Mexico state, Puebla and Chiapas.
Results: The most important findings were:
• 90% of replacements are *M. hyo* positive on arrival.
• 47% of the producers introduce replacements into the acclimation process between 16 and 20 weeks of life.
• 67% use vaccines against *M. hyo* during acclimation, whereas 33% do not vaccinate but introduce the replacements into a positive reproductive herd.
• 27% use cull sows to acclimate.
• 4% use lung homogenate and 10% use piglets as an additional method for acclimation.
• 76% of acclimation sites are continuous flow (CF), and 56% of respondents think that CF can improve the acclimation process.
• Only 68% of those CF quarantines use vaccination protocols against M. hyo.
• 86% do not perform diagnostics to verify an adequate acclimation.
• In 71% of farms the assessment of the stability of *M. hyo* in the reproductive herd is based on clinical signs.
Conclusion: Even though 96% of the respondents consider an adequate acclimation for the control of *M. hyopneumoniae* being important, only 14% of those verify this process. The implementation of a vigilance system in the acclimation process is key in controlling the pathogen to reduce the risk of a possible vertical transmission. This is especially important due to the long persistence of *M. hyo*, which is at least 214 days post infection.

Disclosure of Interest: None Declared

Keywords: Gilts aclimatation , Mycoplasma hyopneumoniae, Survey

Poster Abstracts

Bacteriology and Bacterial Diseases
MYCOPLASMA

PO-PF3-119

Efficacy of a PCV2 and Mycoplasma hyopneumoniae vaccine (Fostera PCV MH) in protecting from M. hyo challenge either 16 or 23 weeks post-vaccination

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Introduction: Conventional *Mycoplasma hyopneumoniae* (MH) vaccines are killed cultures of MH containing whole cells and considerable amounts of extraneous biological material. The novel production process used for Fostera PCV MH (Zoetis) involves cell removal but retains the essential antigens present in the culture supernatant. Studies were undertaken to investigate the duration of MH protection obtained with this vaccine.
Materials and Methods: Two studies of similar design were conducted. In both cases 120 piglets, seronegative for MH (S/P ratio <0.3), were randomly divided into 4 treatment groups of 30 and vaccinated with a single dose of vaccine at 3 weeks of age. Group 1 was given a vaccine containing PCV2 only, groups 2 and 3 were given experimental batches of Fostera PCV MH of different MH content, and group 4 was given another PCV2 MH combination vaccine commercially purchased in the USA. Piglets were maintained free of MH exposure (monitored serologically and by use of sentinel pigs) until experimentally challenged either 16 or 23 weeks post vaccination. Challenge was with a MH-containing lung homogenate administered intra-tracheally. Pigs were slaughtered and necropsied 28 days post-challenge and the % of lung affected by MH lesions scored using a standardized technique by assessors blind as to the treatment group. Impact on lung lesions was the primary study endpoint. Both studies were subject to ethical review and followed all relevant animal welfare requirements.
Results: In the 16 week study % lung lesions were 5.8, 1.6, 1.5 and 2.8 for groups 1 to 4 respectively. All MH vaccine groups were statistically different to group 1 ($P<0.0001$ for groups 2 and 3 and $P=0.0054$ for group 4). The 23 week study was confounded by PRRSv infection post-challenge and the presence of multiple bacterial pathogens in addition to MH. Lung lesions were 14.4, 9.1, 8.3 and 7.1% respectively. All MH vaccine groups were again statistically different to group 1 ($P=0.0466$ for group 2, 0.0214 for group 3 and 0.0036 for group 4).
Conclusion: Lung lesions in the 16 week study were in line with expectations for the model, which typically gives less severe disease as pigs get older. Both batches of Fostera PCV MH showed efficacy, numerically superior to that obtained with another product at commercial potency. Lung lesions in the 23 week study were high in all groups, illustrating the impact of concurrent disease. Nevertheless, all vaccine groups still showed statistically significant reductions in lung lesions indicating an enduring benefit from MH vaccination when challenged 23weeks following vaccination.

Disclosure of Interest: J. Allison Conflict with: Zoetis, G. Nitzel Conflict with: Zoetis, D. Fredrickson Conflict with: Zoetis, V. Rapp-Gabrielson Conflict with: Zoetis, L. Taylor Conflict with: Zoetis, Y. Diamondidis Conflict with: Zoetis

Keywords: Duration, Mycoplasma hyopneumoniae, Vaccine

Bacteriology and Bacterial Diseases
MYCOPLASMA

PO-PF3-272

Molecular characterization of Mycoplasma hyopneumoniae in a multi-site endemic farm by MLVA

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Introduction: Multiple Locus Variable Tandem Repeat Analysis (MLVA) is a useful method to characterize bacterial strains and understand the transmission chains and sources of infection in order to implement more effective control measures. The present study is aimed to use MLVA technique to characterize M.*hyopneumoniae* (M.***hyo***) strains in pigs from wean to finish in the same herd.

Materials and Methods: The study was carried out in a three-site herd in the North of Italy with endemic *M. hyo* infection on both vaccinated and unvaccinated animals. Tracheobronchial swabs (TBS) were taken from each pig at the first week of life (T1) and once a month until 9 months old (T2-T10). During the slaughtering, lungs were inspected and the presence and severity of lesions were recorded. TBS and lung samples were analyzed by qPCR directed against the p102 gene of *M. hyo*. Based on qPCR results, 113 positive samples (74 TBS and 39 lungs) belonging to 18 different animals were characterized by MLVA. The method is based on the detection of the number of tandem repeats at four multiple variable number tandem repeat (VNTR) loci within the genome (Locus 1, Locus 2, p 97-1, p 97-2). The monitoring of M.*hyo* population in this specific herd was further deepened analyzing other 8 samples (7 nasal swabs and two lungs), collected at 1 and 2 years after the study.
Results: Both in vaccinated and unvaccinated animals, M.*hyo* infection was detected by qPCR from T5 but a general decrease was observed at T10. Furthermore, most of the animals showed fluctuating level of M.*hyo* and only few animals were positive throughout the study until the 10th month. Out of 113 specimens 97 samples were completely characterized by MLVA. Two distinct strains (MLVAtype-1 and MLVAtype-2) were identified: MLVAtype-1 was detected in all the analyzed animals from T5 until the last time point and in the lungs; MLVAtype-2 was detected in five pigs. The second genotype was identified especially in the lungs (10) and in TBS collected from two animals at T9 or T10. Furthermore in one animal a coinfection of two different strains in the same lung samples was observed. The 8 samples collected at 1 and 2 years after the study were characterized by MLVAtype-2.
Conclusion: At the beginning of the study a single M.*hyo* genotype was prevalent in the herd infecting all the examined animals. A second genotype, detected at the last time points and in the lungs, could indicate a second wave of infection This second strain seems to persist in the herd as observed during the following analysis. Moreover, the present study evidenced the presence of two different strains of M.*hyo* in the same herd and even in the same animal.

Disclosure of Interest: None Declared

Keywords: molecular typing, Mycoplasma hyopneumoniae, VNTR

Bacteriology and Bacterial Diseases
MYCOPLASMA

PO-PF3-306

The effect of Ingelvac MycoFLEX® sow vaccination on suckling piglets sero-prevalence in a farrow-to finish farm in Taiwan

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Introduction: Mycoplasma hyopneumoniae (Mhyo) causes the enzootic pneumonia in pig. Clinical signs of Mhyo infection including dry cough, retarded growth, and reduced performance result in economic losses. Vaccination is a common approach to control Mhyo in pigs. Sow vaccination is not popular in Taiwan because lacking related information. Lin *et al.* reported sow Mhyo vaccination increased sero-positive rate in sows and piglets [1]. The objective of this study is to evaluate the effect of Ingelvac MycoFLEX® sow vaccination on suckling piglets sero-prevalence in a farrow-to finish farm in Taiwan.
Materials and Methods: This study was conducted in a 500 sow level, farrow-to-finish single site farm which suffered severe respiratory problem in pre-weaning piglets. Early vaccination at 7 days of age against Mhyo was implemented but no significant improvement observed. They decided to try Mhyo sow vaccination after discussion with veterinarian. One batch of piglets from non-vaccinated (NV) and another batch from vaccinated sows (VX) were included in this study. The two batches of sows shared same vaccination/medication program and management, except an additional vaccination of Ingelvac MycoFLEX® (Boehringer Ingelheim Animal Health) 1 dose (1mL) in VX group at 4 weeks pre-farrowing. Serum samples of 10 piglets in each group were collected at 1, 2, 3, 4 weeks of age. Commercial enzyme-linked immunosorbent assay (ELISA) kit (IDEXX M. hyo Ab Test) was used to evaluate sero-prevalence in suckling piglets. The methods and procedures of ELISA test were followed manufacturer's instructions. Boxplot analysis of ELISA titers was done using Minitab 17.
Results: The NV piglets were sero-positive only at 1 week of age with 60% prevalence. The prevalence in VX piglets were 80%, 70%, 50%, and 40% in 1, 2, 3, 4 weeks old groups, respectively (Table 1). Overal, the distribution of Mhyo S/P ratios in VX group was higher than in NV group at all ages investigated in this study (Figure 1). The incidence of respiratory symptoms was reduced in the VX piglets according to the feedback by producer.
Conclusion: Respiratory symptoms in suckling piglets is still a problem in Taiwan, especially in farrow-to-finish single site farm. Some veterinarians suggest early Mhyo vaccination. However, recent studies indicated early Mhyo vaccination resulted in negative impact to pre-weaning piglets such as lower weaning weight [2, 3]. Pre-weaning respiratory problems on piglets maybe due to unstable sow herd under the infection chain thinking. In this study, sow vaccination with Ingelvac MycoFLEX® improved the maternal antibodies. Sow vaccination could be the solution to stable sow herds for producing healthier piglets.

Disclosure of Interest: None Declared

Keywords: Ingelvac MycoFLEX, Sow, Taiwan

Bacteriology and Bacterial Diseases
MYCOPLASMA

PO-PC03-012

Mycoplasma hyopneumoniae load is associated with lung lesion score and pathohistological damage

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Introduction: *Mycoplasma hyopneumoniae* (*M. hyo*) is ubiquitous and world-wide distributed. The agent causes significant losses in production efficacy by mycoplasma-associated pneumonia. However, the direct pathogenicity of the agent is low and its major role is to inhibit clearance and immune system, triggering pneumonia by secondary pathogens. Vaccination against *M. hyo* is widely applied to control the pathogen, but sterile immunity is not realised. Thus, *M. hyo* can routinely be found in pigs with or without pneumonia and an aetiological diagnosis is not easily to achieve. In most cases, clinical pathological, histological and microbiological data are combined, while the demonstration of the pathogen in typical histological alterations by IHC or ISH provides the definite diagnosis. This concept is time-consuming and laborious. Therefore, the availability of a reasonable threshold of bacterial load in affected fluids or tissues by qPCR that could differentiate between colonisation and *M. hyo* associated pneumonia, could be extremely helpful to improve *M. hyo* diagnostics in the future.
Materials and Methods: 155 lungs from pigs of different herds were investigated macroscopically, histopathologically and by the load of M. hyo (qPCR). At the abattoir a lung score was recorded and samples were taken from cranial lobes. If lungs were affected, these regions were preferred.
Results: *M. hyo* load was associated with hyperplasia of the BALT (r=0.41; p<0.001), inflammation in alveolar and bronchiolar lumina (both r=0.31; p=0.003) and lung score (r=0.23; p=0.026), but not with lobular interstitial inflammation. Significant associations between qPCR and histopathology scores provide a simplified and suitable scoring system for the diagnostics of *M. hyo* associated lung damage. A "cut-off" was defined at $5x10^3$ *M. hyo* equivalents per mg of lung tissue. Lung samples with *M. hyo* loads below and over 5×10^3 equivalents showed score confidence intervals (95%) of 1.9-8.1 vs. 7.3-13.1 (lung score; p=0.022), 1.0-1.8 vs. 2.0-2.5 (inflammation in bronchiolar lumina; p<0.001) and 1.4-2.3 vs. 2.5-3.0 (BALT-hyperplasia; p<0.001), respectively.
Conclusion: Although differences in histopathology and lung score between samples with less or more than 5×10^3 *M. hyo* equivalents per mg of lung tissue were statistically highly significant, a residual variability remained within both groups. Thus, this threshold cannot be applied on an individual animal basis. Further studies and the inclusion of strain and damage related sequence information, e.g. by multiplex qPCR and the consideration of other respiratory pathogens might help to rule out the suitability in future field diagnostics.

Disclosure of Interest: None Declared

Keywords: diagnostics, qPCR

Poster Abstracts

Bacteriology and Bacterial Diseases
MYCOPLASMA

PO-PF3-280

Laboratory results of swine samples from wean to finish pigs with respiratory symptoms

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Introduction: Respiratory diseases are commonly found in wean to finish pigs. The causative pathogen of these diseases is hard to diagnose only by clinical observations and macroscopic lesions. Therefore, in order to determine the etiologic agents involved in the infection, basic and complementary diagnostic tools are required.
In many cases, early diagnosis errors contribute to ineffective action plans resulting in increased costs of production.
This article summarizes laboratory results of animal samples sent in from all regions of Brazil.
Materials and Methods: These data refer to samples from 64 animals out of 21 herds with respiratory symptoms between 90 and 170 days of age, undergoing diagnostics in the reference diagnostic centers in Brazil during the year 2015 (unpublished data). The tests used were histopathology, immunohistochemistry (IHC), PCR and bacterial isolation.

Results: The results of the histopathological analysis, IHC, bacterial isolation and PCR demonstrate the involvement of several infectious agents in most cases of respiratory problems in pigs. Of the 64 cases investigated, 14 cases (21.8%) showed lesions suggestive for *Mycoplasma hyopneumoniae* (Mhyo). In 20 cases (31.2%) lesions were found suggestive for Influenza virus. An association of Mhyo with Influenza virus was found in 6 cases (9.3%). Characteristic lesions involving Influenza virus in combination with other bacteria were found in 9 cases (14%). Bacteria were found in 13 cases (20%), and involvement of Circovirus in 2 cases (3%).
Conclusion: Our results demonstrate that the major infectious agent involved in respiratory symptoms is Influenza virus. Alone or in combination with other bacteria, Influenza was found in 54% of cases analyzed. This demonstrates the need for targeted laboratory investigation to confirm the clinical suspect, since most of the cases were presented as *Mycoplasma hyopneumoniae* prime suspects.
Diagnostic tools and correct interpretation of laboratory results are of special importance in the improvement of the professional pig farming. Most respiratory diseases have a complex etiology in which the correct diagnosis will depend on the targeted use of different diagnostic tools, associating history of the herd and results from clinical examination, necropsy and laboratory analysis.
Professionals working in the field perform many activities related to clinical and pathological diagnosis, like collecting material for laboratory tests. The training of veterinarians to implement the diagnostic tools and methodologies available is very important to achieve accurate results.

Disclosure of Interest: None Declared

Keywords: Diagnostic, respiratory disease, swine

Bacteriology and Bacterial Diseases
MYCOPLASMA

PO-PF3-053

Colorimetric Method For Determining Viability And Antibiotic Susceptibility For Porcine Mycoplasma Strains Using A Redox Indicator

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Introduction: *Mycoplasma hyopneumoniae* (Mhp), *Mycoplasma hyorhinis* (Mhr), and *Mycoplasma hyosynoviae* (Mhs) have caused a big economic loss in the pig industry worldwide. Recently the emergence of multidrug–resistant strains of mycoplasma species has become more common. In order to obtain the antibiotic susceptibility of each field strain a colorimetric method using alamarBlue assay was adopted to determine the viability of tested culture. If mycoplasma is alive after incubation with an antibiotic, the innate metabolic activity of mycoplasma will make alamarBlue in reduced form (pink). Otherwise, alamarBlue will be in the oxidized form (blue).
Materials and Methods: Tested cultures were adjusted to 10^8 CCU/ml for Mhp and to 10^8 CFU/ml for Mhr and Mhs. Each of the thirteen sterile tubes was added 0.9 ml of Friis broth (FB) and 0.1 ml of tested mycoplasma culture. Each tube from Tube one through Tube twelve was labeled with the name of one of the twelve antibiotics (BD sensi-Disc) and added with a piece of the corresponding antibiotic disk. Tube thirteen was not added with any disc and used as a positive control. Tube fourteen was added with 1 ml of FB and 0.1 ml of inactivated culture and used as a negative control. Each tube was then added with 0.1 ml of alamarBlue (life Technologies) and incubated at 37°C for 3 days. Aseptic procedure was followed in order to avoid bacterial contamination. Antibiotic was tested at 2 IU/ml (Penicillin), 30 ug/ml (Oxytetracycline), 30 ug/ml (Tetracycline), 30 ug/ml (Tiamulin), 5 ug/ml (Enrofloxacine), 23.75 ug/ml (Sulfamethoxine) and 1.25 ug/ml (Trimethoprim), 20 ug/ml (Amoxicillin), 30 ug/ml (Tulathromycin), and 15 ug/ml (Tilmicosin) respectively. The color change and OD_{630} reading of each sample were recorded. A broth dilution technique was also set up to determine minimum inhibitory concentration (MIC) of antimicrobial agents against mycoplasma strains using alamarBlue.
Results: In this study it was found that antibiotic susceptibility of mycoplasma strain can be obtained using antibiotic sensi-disc or broth dilution technique if alamarBlue was used as an indicator. In this study some fifty porcine mycoplasma field strains were tested for their in vitro susceptibility to twelve antimicrobial agents.
Conclusion: In conclusion, this study is the first description of an in vitro antibiotic susceptibility test for porcine mycoplasma strains using a redox indicator.

Disclosure of Interest: None Declared

Keywords: mycoplasma

Bacteriology and Bacterial Diseases
MYCOPLASMA

PO-PF3-107

Monitoring of antimicrobial susceptibility of Mycoplasma hyopneumoniae isolated from respiratory tract infections in pigs across Europe during 2010-13

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Introduction: MycoPath is the first ongoing European resistance monitoring program for *Mycoplasma* pathogens isolated from diseased but not yet treated cattle, pigs and poultry. Antimicrobial susceptibilities of *M.hyopneumoniae* pathogens isolated from pigs suffering from respiratory disease are presented here.
Materials and Methods: Lung samples were collected post-mortem from pigs with clinical signs of enzootic pneumonia (EP) or from abattoir-slaughtered pigs with EP lung pathology from known endemically-infected herds. Samples were collected from animals that had not received recent antibiotic treatment (>15 days), in Belgium (BE), Spain (E) and UK. *M.hyopneumoniae* strains were isolated by national laboratories (only one isolate per outbreak/farm retained). Susceptibility to nine veterinary-use antibiotics was determined in a central laboratory by broth microdilution methodology. Results are presented as MIC ranges and $MIC_{50/90}$ (in µg/ml). MIC values of the 3 countries were statistically compared with the Mann-Whitney test.
Results: Fifty isolates were obtained: 16 from BE, 14 in E and 20 in UK. Similar MIC ranges were determined for enrofloxacin (0.008-1) and marbofloxacin (0.002-1) with $MIC_{50/90}$ values of 0.031/0.5. The macrolide antibiotics displayed overall slightly lower $MIC_{50/90}$ values: 0.062/0.25 for spiramycin, ≤ 0.001/0.004 for tulathromycin and 0.031/0.125 for tylosin. MIC ranges were as follows: 0.008-0.5 (spiramycin), ≤0.001-0.016 (tulathromycin) and 0.004-0.5 (tylosin). For spiramycin and tylosin MICs assessed for BE and UK were significantly higher than those for E. Low MIC ranges and $MIC_{50/90}$ values were found for both pleuromutilin antibiotics: MIC range of 0.002-0.125 and $MIC_{50/90}$ 0.016/0.062 for tiamulin and MIC range ≤0.001-0.002 and $MIC_{50/90}$ ≤0.001/≤0.001 for valnemulin. Similar valnemulin $MIC_{50/90}$ values were determined for all three countries but tiamulin MIC values were found significantly higher for UK in comparison to E. The florfenicol $MIC_{50/90}$ values were numerically slightly higher in UK (0.5/1.0) in comparison to BE or E (0.25/0.5). MIC range for oxytetracycline was ≤0.001-2.0 with $MIC_{50/90}$ values of 0.062/0.25. In the absence of validated standards and clinical breakpoints, it is currently not possible to reliably extrapolate these *in vitro* results towards *in vivo* efficacy. This is problematic for veterinary practitioners who need to decide on the most appropriate treatment of diseased animals.
Conclusion: This project provides valuable information on *in vitro* antimicrobial susceptibility of European *M.hyopneumoniae* isolates, highlighting the urgent need for *Mycoplasma*-specific laboratory standards and interpretive criteria.

Disclosure of Interest: None Declared

Keywords: Antimicrobial susceptibility, EU, M.hyopneumoniae

Bacteriology and Bacterial Diseases
MYCOPLASMA

PO-PF3-248

Mycoplasma hyopneumoniae surveillance – a production system approach for health management decision making

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Introduction: *Mycoplasma hyopneumoniae* (*M.hyo*) is a common etiologic agent that causes economic losses in all production stages of our system. Despite the clear negative clinical impact, the pathogenesis of disease is unknown and variable across the 38 sow flows. The goal in this project was to develop a surveillance program to successfully classify the *M.hyo* status and strain within three distinct production flows. By routinely testing the gilt development units (GDU), sow farms, and downstream pigs of each flow, we can determine the timing of *M.hyo* colonization and infection dynamics within the system to develop systematic intervention methods to reduce or eliminate the clinical impact.
Materials and Methods: Historical wean-to-finish (wtf) diagnostics identified three sow farms experiencing clinical *M.hyo.* Each sow farm represents one of three distinct genetic flows. At each sow farm, 30 laryngeal swabs were collected from gilts entered into the sow farm 8 weeks prior from the GDU, at expected peak *Mhyo* shedding, to prove *M.hyo* instability. Swabs were tested individually by real-time PCR at the BIVI HMC, Ames, IA. Positive PCR samples were sent to the University of Minnesota VDL for sequencing and a comparative dendrogram developed. After baseline sow farm sampling, additional laryngeal swabs were tested at the gilt grow-finish, GDU, and wtf at approximately 8 weeks placed.
Results: Gilts in Flow A tested negative for *M.hyo* at the grow-finish and GDU. However, 27% of gilts tested positive at the sow farm. No wtf pigs have tested positive for *M.hyo*, but sampling is ongoing. **Gilts in Flow B** also tested negative for *M.hyo* at the grow-finish and GDU. Again, 27% of gilts tested positive at the sow farm, and 24% of wtf pigs were positive. **Gilts in Flow C** tested negative for *M.hyo* in grow-finish, but 40% of gilts tested positive at the GDU. The sow farm and wtf both tested negative. Sequence results showed each multiplication flow has a unique *M.hyo* strain with 99%+ homology consistent across each production stage. Overall, the three flows are 97% homologous, but each flow has a distinct dendrogram cluster.
Conclusion: The results have led to discussion about *M.hyo* stabilization and elimination programs. The keys to successful implementation will be early gilt acclimation, followed by a 250+ day cool down to clear active infection before first farrowing. The previously described surveillance strategy utilizing laryngeal swabs will be used to confirm the cessation of *M.hyo* shedding prior to gilt entry into the sow farms. Sequencing and dendogram development have been helpful epidemiological tools that allow agent tracking and support the system approach.

Disclosure of Interest: None Declared

Keywords: Gilts, Mhyo, Surveillance

Poster Abstracts

Bacteriology and Bacterial Diseases
MYCOPLASMA

PO-PF3-221

PORCILIS® PCV M HYO VACCINATION IN FRANCE: A FIRST ASSESSMENT OF LUNG LESIONS AT SLAUGHTERHOUSE

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Introduction: *Mycoplasma hyopneumoniae* (MH) and Porcine Circovirus type 2 (PCV2) are two important pathogens that cause economic losses in fattening pigs. Early 2015, MSD AH introduced Porcilis® PCV M Hyo (PCVM), a new MH and PCV2 single dose ready to use vaccine for piglets. Early 2015, a significant number of "SELAS Vétérinaire de la Hunaudaye" customers began vaccination with PCVM. An accurate follow-up was carried out and the first results are presented here. The purpose of this study was to compare lung lesion scores of PCVM vaccinated batches with those of batches that received the previous vaccination program.
Materials and Methods: The thirthy one (31) farms included in the study had a slaughter check done between Jul-Sep 2015 on a PCVM vaccinated batch ("PCVM controls") and in May-Jun 2015 on a batch vaccinated with the prior protocol ("BeforePCVM controls"). To exclude any difference due to other factors, farms with unchanged vaccine protocol were included randomly in the study. Fifty seven (57) farms ("SummerCtrl group") were checked in the same period of "PCVM controls" and 42 farms ("SpringCtrl group") were inspected at the same time as "BeforePCVM controls".
All slaughter checks were performed using Madec quantitative lung scoring method (0-28). Average lesion score, percent of healthy lungs and highly affected lungs (score ≥8/28) were recorded for each check. IFIP pleurisy scoring system (0-4) was adopted for pleurisy lesions. Average score and percentage affected lungs were recorded. IFIP rhinitis scoring system (0-20) was used for nasal lesions. Average lesion score and percentage of healthy noses were recorded. The percentage of livers with "milk spots" was also considered.
Statistical analyses were performed with Z Test (p =0.05).
Results: Lung and nasal lesions were not significantly different between "PCVM controls" and "BeforePCVM controls" or between seasonal control groups. There was a significant difference in pleurisy lesions between "PCVM controls" and "BeforePCVM controls" (p<0,02) and between seasonal control groups (p<0,01). Pleurisy average score and percentage were greater in spring slaughter checks than in summer ones.
Conclusion: The 31 farms that implemented Porcilis® PCV M Hyo vaccination remained stable compared to the previous vaccination program in terms of lung and nasal scores. Next, a new series of slaughter checks is scheduled for the same 31 farms to compare scores six months after starting vaccination with Porcilis® PCV M Hyo.
This study also showed seasonal variations of pleurisy scores that absolutely must be taken into account in the case of vaccination protocol change.

Disclosure of Interest: None Declared

Keywords: Porcilis PCV M Hyo, Slaughterhouse, Vaccination

Bacteriology and Bacterial Diseases
MYCOPLASMA

PO-PF3-303

Field efficacy of ZACTRAN® (gamithromycin injectable solution) for the treatment of Mycoplasma hyopneumoniae for swine in Japan

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Introduction: Porcine respiratory disease complex is a widespread and complex condition involving viral agents and bacterial agents of which Mycoplasma hyopneumoniae (M. hyo), the etiologic agent of enzootic pneumonia, may play an important role. Macrolides are long-lasting antimicrobials which accumulate in the lung. Gamithromycin, a novel azalide (macrolide subclass) could thus be an appropriate tool for the treatment of diseases caused by M. hyo.
Materials and Methods: This study was conducted on 2 farms located in Japan, previously qualified for the study by random necropsy of sentinel pigs and microbiological identification of M. hyo.A total of 120 1.3 to 2-month-old pigs, healthy other than demonstrating clinical signs of SRD (swine respiratory disease), were randomly assigned to receive either a single intramuscular injection of ZACTRAN® (gamithromycin injectable solution, 6.0 mg/kg bodyweight) or sterile saline. On each site, the experimental pigs were contemporaneous and from the same farrowing batch as the sentinels.Pigs were observed daily for any abnormality during the course of the study. Individual bodyweights (BW) and morbidity signs evocative of SRD were recorded on D0, D30, D60 and D90. Ten and 30 pigs per group were euthanized on D7 and D90 respectively, for necropsy. Lungs were carefully inspected for lung hepatization lesion scoring and area calculation. Any animal that died was necropsied as well to determine the cause of the death.Post-treatment BW curves and daily gains (BWG) were compared by fitting a mixed model. Lung hepatization on D7 and D90 was compared between treatment groups using the van Elteren rank test.
Results: No adverse event was observed after injection in ZACTRAN-treated pigs. Post-treatment BW and BWG was significantly higher in the ZACTRAN-treated group than in the saline-treated group (p<0.001 for all time points). Lung hepatization score in ZACTRAN-treated group was significantly lower on D7 and D90 (p=0.013 and p<0.001, respectively). Average lung hepatization area in the ZACTRAN-treated group was significantly lower than in the saline-treated group on Day 90 (p<0.001). A total of 43% of the saline-treated pigs versus 2% of the ZACTRAN-treated pigs showed clinical signs evocative of SRD. Mortality due to SRD was 15% in the saline-treated group and 7% in the ZACTRAN-treated group.
Conclusion: Under the conditions of this trial, pigs with spontaneously acquired *Mycoplasma hyopneumoniae* infection administered a single intramuscular injection of ZACTRAN at 6 mg/kg showed better weight gain, lower morbidity and mortality due to respiratory disease and reduced lung hepatization than the saline-treated control pigs.
®ZACTRAN is a registered trademark of Merial, Inc.

Disclosure of Interest: Y. Kondo Conflict with: Merial Japan Limited, N. Nakanishi: None Declared, Y. Wakui Conflict with: Merial Japan Limited, A. Richard-Mazet Conflict with: Merial S.A.S., G. Kinoshita Conflict with: Merial Japan Limited, P. Jeannin Conflict with: Merial S.A.S.

Keywords: Gamithromycin, Mycoplasma hyopneumoniae

Bacteriology and Bacterial Diseases
MYCOPLASMA

PO-PF3-279

Efficacy comparison following pig vaccination with either Porcilis PCV M Hyo or a mixed combination vaccine against PCVAD and Mycoplasma hyopneumoniae

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Introduction: PCV and Mhyo piglet vaccination rate in The Netherlands is high and has further increased after registration of a PCV2 and Mhyo vaccine that is mixed prior to vaccination. Recently, Porcilis®PCV M Hyo was introduced as the first RTU PCV2/Mhyo vaccine in the EU. In order to choose the most effective PCV and Mhyo piglet vaccine, efficacy, safety and convenience was evaluated in a comparative trial in a Dutch closed pig farm with PCVAD and Mhyo especially in the finishing period.
Materials and Methods: The trial farm kept 170 sows in a 2 week batch system. PCVAD was characterized by decreased average daily weight gain (ADWG) after stopping piglet PCV2 vaccination. Slaughterhouse results revealed Mhyo like lesions in 22% of pigs. Serology and PCR testing confirmed PCV2 infection at the end of the nursery period.
Three (3) week old piglets from 6 batches were randomly allocated to 3 groups and were vaccinated: P-Porcilis PCV M Hyo (n=266), X-mixed combo (n=262), C-saline controls (n=268). Pigs from the different groups were commingled throughout the study.
Pigs were individually weighed at 3, 10, 18 and 22 weeks of age to calculate ADWG (gr/day). Possible side effects were monitored at vaccination and 1 hour later, and investigator and farmer were asked to give their opinion about the convenience (time to prepare vaccine, syringeability and hygiene). Mortality was recorded. Mhyo efficacy was evaluated by scoring the % of M hyo like lesions and reduced severity (MADEC system).
Results: There were no post-vaccination side effects in any of the groups and no ADWG differences at end of the nursery phase (10 wks of age). During finishing (10-22wks), ADWG significantly increased in vaccinated pigs (P: 842, X: 827) compared with controls (C: 803). Mortality between 3 and 22 wks was 5.5% (C), 4.5% (X) and 3.3% (P). There were numeric differences in % Mhyo lesions at slaughter between the treatment groups (C: 12.6% (23/182); X: 7.4% (12/163); P: 6.3% (11/173). Severity of Mhyo lesions was significantly lower than controls in group P only. Farmer and investigator declared that saline and P-injections were most convenient with respect to preparation to injection time and hygiene (no mixing before injection).
Conclusion: Both vaccines were safe due to absence of local and systemic reactions and differences in ADWG up to 10 weeks of age. ADWG was higher in the finishing period for both vaccine groups compared to the controls, but was best for Porcilis®PCV M Hyo. In addition, Porcilis®PCV M Hyo RTU vaccination proved to be safe and effective against both PCVAD and Mhyo, and was also more convenient because a mixing step is not needed.

Disclosure of Interest: None Declared

Keywords: comparison, Mhyo lesions, RTU vaccination

Bacteriology and Bacterial Diseases
MYCOPLASMA

PO-PF3-039

Mycoplasma hyosynoviae infections in finisher pigs with arthritis

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Introduction: Since 2001 GD Animal Health in the Netherlands runs a monitoring system on pig health. One pillar of this system is the recording of all telephonic questions concerning health issues. Since 2013 lameness turned out to be the main subject of these questions. A detailed telephonic survey in 2014 revealed that most of the questions on lameness concerned finisher pigs between 3 and 5 months of age. The symptoms reported were acute lameness in more than one leg and swollen joints and bursas. However, rectal temperature was normal and mortality low. Predominantly hindquarters were affected. According to literature these symptoms are associated with a *Mycoplasma hyosynoviae* (*M. hyosynoviae*) infection. However, in the Netherlands, this pathogen is rarely confirmed in pigs. Another possible cause might be osteochondrosis or a combination of both. In order to establish whether *M. hyosynoviae* plays a role in the described problems, selected pigs submitted for post mortem investigation were specifically examined.
Materials and Methods: Pigs, between 3 and 5 months of age, submitted for post mortem investigation to the GD Animal Health laboratory, with a history of acute lameness and swollen joints were selected for further investigation. Samples were taken from the affected joints and sent to the laboratory of the Tierärtzliche Hochschule in Hannover, Germany to demonstrate *M. hyosynoviae* by culture. Joint tissue and adjacent bone tissue were also assessed histologically for signs of inflammation and osteochondrosis.
Results: In the period June until December 2015 19 pigs (from 10 herds) with lameness were investigated pathologically. From 36 joints a smear was cultured. *M. hyosynoviae* was detected in 8 joints (6 pigs from 4 herds), *Streptococcus suis* in 7 joints (3 pigs / 2 herds), mixed culture in 6 joints (5 pigs / 4 herds) and no bacteria were found in 7 joints (6 pigs / 4 herds). Results form 8 joints (3 pigs / 2 herds) are pending. All joints with an *M. hyosynoviae* infection showed indications of osteochondrosis and all but one also presented clear signs of arthritis consistent with a *M. hyosynoviae* infection.
Conclusion: From the results of this limited survey it can be concluded that *M. hyosynoviae* most likely plays a significant role in acute lameness of finishers pigs between 3 and 5 months of age in the Netherlands. Osteochondrosis might be a cofactor in the pathogenesis. However, further investigation is needed to clarify the role of *M. hyosynoviae* and to establish appropriate preventive measures.

Disclosure of Interest: None Declared

Keywords: Mycoplasma hyosynoviae, osteochondrosis, arthritis

Poster Abstracts

Bacteriology and Bacterial Diseases
MYCOPLASMA

PO-PF3-008

Use of stochastic modeling to determine number of laryngeal swab sample pools and collections for detection of low Mycoplasma hyopneumoniae prevalence

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Introduction: There is a need for ante-mortem *Mycoplasma hyopneumoniae* (Mhp) diagnostic sampling protocols to determine if populations are negative (≤1% prevalence (Pr)), remain negative over time, and to detect early infection to prevent spread. A recent study showed that increased sample size resulting from ante-mortem laryngeal swab sampling (LS) combined with pooling allowed for a higher herd detection rate by PCR while pursuing the most economical approach, in a high Pr population. The following study was designed to determine ante-mortem LS guidelines for detection of Mhp by PCR at low Pr levels.
Materials and Methods: A stochastic sampling model was used to determine the number of pigs and the number of times to sample herds in order to detect one positive pig in a pool when the rest of the pool was negative in a Mhp low Pr and high Ct scenario. DxSe have been described previously for a high (H=36) Ct in 3:1 pools and 5:1 pools. Three DxSe were run for 3:1 pools: 79.1% (99% lower confidence limit (LCL)), 81.9% (95% LCL), and 90% (mean) and 5:1 pools: 58.5% (99% LCL), 61.8% (95% LCL), and 72% (mean). For each adjusted DxSe value, the model was run for the number of individuals sampled from a 2,500 population size (30, 60, 90, and 120) and a percent Pr (1%, 2%, 3%, 4%, and 5%). For each pool, adjusted DxSe, N individuals and percent Pr, two values were recorded from the stochastic model: a detection probability of ≥99% and a detection probability of ≥95%. For each detection probability, a minimum of 100 iterations were run and the highest value, indicative of the number of collections needed, was recorded.
Results: Using 99% LCL and 95% detection probability, the number of sample collections required for detection of 1%, 2%, 3%, 4%, or 5% Mhp group Pr decreases as Pr increases and as the number of individuals sampled increases to 30 (15, 8, 5, 4, or 3 collections), 60 (8, 4, 3, 2, or 2 collections), 90 (6, 3, 2, 2, or 2 collections), and 120 (4, 3, 2, 2, or 2 collections) for pools of 3. Similarly, for pools of 5, the number of sample collections required decreases as Pr increases and as the number of individuals sampled increases to 30 (21, 11, 7, 5, or 4 collections), 60 (11, 5, 4, 3, or 3 collections), 90 (7, 4, 3, 2, or 2 collections), and 120 (6, 3, 2, 2, or 2 collections). Additional scenarios will be available on a future website.
Conclusion: These novel Mhp sampling guidelines take into account DxSe for the LS procedure and provide guidance for determining number of pigs and of samplings required to economically detect Mhp in low prevalence scenarios. These guidelines are actively being implemented to monitor Mhp suspected negative populations.

Disclosure of Interest: None Declared

Keywords: laryngeal swab, Mhyo, stochastic models

Bacteriology and Bacterial Diseases
MYCOPLASMA

PO-PF3-172

Can Lung Lesions Scoring Be Replaced by Monitoring M hyo antibodies?

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Introduction: Lung Lesion Scoring (LLS) is the method of choice for checking diseases like enzootic pneumoniae (EP) and for analyzing the effect of intervention schemes. However, LLS is a time consuming process and there are situations where performing LLS in a group of market pigs is not possible. Furthermore, LLS determination in a slaughterhouse is not suitable for routine monitoring. Serological investigation is more practical for routine monitoring but serological investigation for M hyopneumoniae (M hyo) has drawbacks. When a relationship can be established between EP-LLS and serology (S/P ratio) this will have both practical and financial advantages. M hyo serology is included in most herd monitoring schemes. Both the detection of a M hyo infection and information on the severity of lesions induced by the infection is important.
Materials and Methods: In batches of 100 pigs from 71 farms the EP-LLS was determined using the Madec and Kobisch method. From every batch 20 serum samples were collected and analyzed for M hyo antibodies with the BioChek M hyo ELISA test kit. Statistical methods were used to detect the relationship between the BioChek M hyo S/P ratio and farms with low or high LLS scores. The mean S/P ratio of a batch of serum samples and the average LLS of that farm were also investigated. In a different setup 66 pigs were followed chronologically in time.
Results: After an M hyo infection a correlation on farm level between the BioChek M hyo ELISA S/P ratio and the LLS was found. Farms (n=27) with a higher EP-LLS score had a higher mean S/P ratio (1.26 ±0.71). Farms with a lower LLS score (n=44) had a lower mean S/P ratio (0.90±0.91). When all pigs were included the R value was 0.38 (P=0.003). When only the pigs with an LLS were included the R value was 0.36 (P = 0.007).
In the chronological study containing 66 pigs a R=0,67 (P = 0.008) was found. The S/P ratio found at 18 weeks of age would explain 47% of the recorded LLS.
Conclusion: There is no correlation between M hyo serum antibodies and clinical protection. The LLS is an excellent method to monitor respiratory diseases including EP like lesions. The M hyo ELISA is a cheap and fast method to obtain information on the presence of antibodies and their titer. Routine serological monitoring is more and more practiced on swine farms. When monitoring includes M hyo, the resulting BioChek M hyo ELISA S/P ratio will give an indication of the LLS in that batch of pigs. The higher the S/P ratio the higher the EP like LLS. Further investigations will be required.

Disclosure of Interest: None Declared

Keywords: BioChek , monitoring , Mycoplasma hyopneumoniae

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MYCOPLASMA

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Influence of pooling on artificial laryngeal swab sample PCR results in low Mycoplasma hyopneumoniae prevalence scenarios

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Introduction: A recent study comparing ante-mortem versus post-mortem samples by individual and pooled samples for detection of *Mycoplasma hyopneumoniae* (Mhp) by PCR suggested that utilizing pooling and increasing sample size via ante-mortem laryngeal swab sampling (LS) allows for a higher herd detection rate while pursuing the most economical testing approach in a high prevalence population. The following study was designed to determine the extent pooling samples from an artificially created low Mhp prevalence ante-mortem population scenario lowers test diagnostic sensitivity (DxSe).
Materials and Methods: Due to the power required for the study, artificial positive and negative samples comparable to those found in low Mhp prevalence scenarios were created. Treatment groups were chosen for the study based off of a histogram created from field LS results. To mimic LS, an artificial Mhp positive stock solution for each treatment group was created from Mhp strain AP 414 (Ct 20) stored at the University of Minnesota, PBS, and known Mhp negative oral fluids. Dilutions were made to create the desired Ct value for each treatment group: low (L=26), middle (M=31), and high (H=36). Artificial negative samples were made using PBS and known Mhp negative oral fluids. L, M, and H treatment groups were tested in 3:1 and 5:1 pools. The remaining samples in each pool were composed of the known negative samples. Ninety PCR tests (VetMAX™-Plus qPCR Master Mix) were run for each pool and Ct value for a total of 540 PCR tests. Six PCR plates were used to test all of the samples. Fifteen PCRs for each Ct value and pool were run per plate. To reduce potential variation, one technician was responsible for all extractions (MagMAX-96 Viral RNA Isolation Kit) and the amplification process. All PCRs were tested on one day using 3 machines (2 plates/machine).
Results: L and M had similar DxSe results for pools of 3:1 (100% and 98.89%) and 5:1 (100% and 100%) and were more sensitive than H pools of 3:1 (90%) and 5:1 (72.65%). 95% and 99% confidence intervals (CI) were determined for H pools of 3:1 (81.9-95.3 and 79.1-96.4) and 5:1 (61.8-81.1 and 58.5-83.5).
Conclusion: As expected in a low Mhp prevalence and high Ct value scenario, there is a risk of not detecting one positive animal in a pool when the rest of the pool is negative. DxSe and CI obtained from this study have been used in a stochastic model to develop novel LS tables to provide veterinarians guidance in determining number of animals along with number of times to sample herds to most economically detect Mhp in low prevalence scenarios.

Disclosure of Interest: None Declared

Keywords: laryngeal swab, Mhyo, Pooling

Bacteriology and Bacterial Diseases
MYCOPLASMA

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Mycoplasma hyopneumoniae detection and antibody response among various gilt developing units

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Introduction: *Mycoplasma hyopneumoniae* (*M. hyop*) is the etiologic agent of enzootic pneumonia and causes important economic losses to the swine industry. Currently, little information is available on the dynamics of *M. hyop* detection among gilt developing units (GDUs). Therefore, the aim of the study was to evaluate *M. hyop* detection and antibody response among different GDUs within a production system.
Materials and Methods: A cross-sectional study was conducted in 4 *M. hyop* positive multiplier GDUs. In this production system, gilts arrive at the GDUs at 10 weeks of age, are vaccinated at 16 weeks of age and 4 weeks prior to leaving the GDU, and enter a sow farm at ≥30 weeks of age. A total of 150 laryngeal swabs (LS) and paired serum samples were collected from 30 gilts per age group (20, 24, 28, 32, and 36 weeks) for each of the 4 GDUs. LS were pooled per age in groups of 5 and tested by *M. hyop* real-time PCR. Serum samples were individually tested for *M. hyop* antibodies using Idexx ELISA.
Results: *M. hyop* was undetected in LS from 20 and 24 week old gilts until 32-36 weeks of age within 3 of the 4 GDUs. Gilts housed in one GDU were positive for *M. hyop* across all ages except at 24 weeks of age. Three GDUs sourced from the same site varied in the dynamics of *M. hyop* infection due to differences in the initial and continued detection of *M. hyop* in LS across ages and GDUs. Within each GDU, the percentage of gilts with high S/P values potentially suggestive of a recent infection varied across all ages. In 3 GDUs, 20 and 24 week old sampled gilts were negative for *M. hyop* in LS but had high levels of S/P. For each GDU, the percentage of *M. hyop* positive gilts detected using LS did not correlate with the percentage of gilts having high S/P values.
Conclusion: When comparing the 4 GDUs, variability was observed among the age at which gilts initially become infected with *M. hyop*. However, high S/P values in antibodies potentially suggestive of infection were observed upon entry into the GDU. Although gilts originated from positive sources, not all gilts appeared to be exposed at early ages. All gilts sampled across all GDUs were detected positive at 32-36 weeks of age and potentially shedding *M. hyop* upon entry into the sow farm. This further supports information specifying gilts as one of the most important risk factors for *M. hyop* introduction within a sow herd and the importance of gilt acclimation to *M. hyop* at the source site. S/P values compared to LS did not seem to be a good indicator of *M. hyop* shedding and could be misleading due to vaccination.

Disclosure of Interest: None Declared

Keywords: GDU, laryngeal swab, Mycoplasma hyopneumoniae

Poster Abstracts

Bacteriology and Bacterial Diseases
MYCOPLASMA

PO-PF3-287

INVESTIGATION ON THE SEASONALITY OF ENZOOTIC PNEUMONIAE-LIKE LESIONS IN PIGS AT SLAUGHTER IN NORTHERN ITALY

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Introduction: *Mycoplasma hyopneumoniae* (*Mhyo*) is the primary pathogen of enzootic pneumonia (EP), a chronic respiratory disease in pigs causing major economic losses to the pig industry worldwide. Gross lesions compatible with EP are typically observed as grey to purple consolidation of the cranial ventral portions of the lungs during abattoir inspections. Moreover, a seasonal effect on *M.hyo* dynamic of infection has been described before. This study aims to investigate the effect of the season on the prevalence of lung lesions in pigs at slaughter in Northern Italy.

Materials and Methods: A total of 632 batches of finishing pigs from farms located in Northern Italy were studied. Pigs were sent to slaughter to a specialized pig abattoir between June 2014 and May 2015. Lung lesions were assessed and EP-like lesions scored following the methodology described by Madec et al. (1982). The economic impact of *Mhyo* based on EP-like lesions was calculated according to Straw et al. (1989). Abattoir assessments were categorized according to the season of the year that were conducted to investigate seasonal influence on EP-like lesion scores at slaughter. The influence of climatic conditions at placement of pigs in the finishing unit was investigated. The typical finishing period under Italian pig production conditions goes from 30 kg to 170 kg on average and takes about 6 months.
Results: The average EP-like lesion score for the 632 batches was 1.9. When different seasons were studied, significant differences ($p<0.005$) were shown between EP-like lesion scores in summer (1.7) and those recorded in winter (2.1) and spring (1.9). No differences were found between autumn and other seasons. The economic loss associated with *Mhyo* was significantly lower ($p<0.005$) in summer (2.4 €) than in spring (3.3 €) and winter (3.6 €). Lung lesions observed in summer (4.6%) were significantly lower ($p <0.001$) than in winter and spring (5.8% and 5.7%, respectively). Regarding climatic conditions at placement of pigs, we found that atmospheric temperature was positively correlated with EP-like lesion scores and economic impact. Temperature fluctuations between day and night or humidity at placement were not correlated.
Conclusion: This study indicates that, under the conditions of Italian pig production, the prevalence and severity of EP-like lesion at slaughter are affected by the season of the year, decreasing in summer and increasing in winter and spring. Whereas the placement of pigs in the finishing unit over winter may facilitate the spread and severity of *Mhyo*, lung lesions may resolved by the time pigs are slaughtered and its importance may be underestimated.

Disclosure of Interest: None Declared

Keywords: None

Bacteriology and Bacterial Diseases
MYCOPLASMA

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ENZOOTIC PNEUMONIAE-LIKE LESIONS IN PIGS AT SLAUGHTER IN NORTHERN ITALY AND THEIR ASSOCIATED ECONOMIC IMPACT

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Introduction: *Mycoplasma hyopneumoniae* (*Mhyo*) is the primary pathogen of enzootic pneumonia (EP), a chronic respiratory disease in pigs causing major economic losses to the pig industry worldwide. Gross lesions compatible with EP are typically observed as grey to purple consolidation of the cranial ventral portions of the lungs during abattoir inspections, thus being useful for monitoring farm health status and for epidemiological studies. This study aims to investigate the prevalence and severity of EP-like lung lesions in pigs at slaughter in Northern Italy.
Materials and Methods: A total of 120,000 finishing pigs from farms located in Northern Italy were investigated in this study. Pigs (average weight 170 kg) were sent to slaughter to a specialized pig abattoir in 896 batches between February 2014 and August 2015. Lung lesions were assessed in the processing line and EP-like lesions scored following the methodology described by Madec (1982). For each batch, the average EP-like lesion score was calculated. The economic impact of *Mhyo* based on EP-like lesions was estimated according to Straw et al., (1989). For that estimation, an average daily gain (ADG) of 700 g/d, a feed conversion rate (FCR) of 3.5 and 180 days of fattening period were considered. Pig live weight reference prices were obtained from a weekly bulletin.
Results: EP-like lesions were detected in pigs from all studied batches, with an average of 5.3% of the lung tissue affected and values ranging from 1.33% to 23.5% when all batches were considered. The average EP-like lesion score for a batch was 1.85, with a minimum batch score of 0.07 and a maximum of 7.36. The economic losses due to *Mhyo* were estimated in 3.03 €/pig on average, ranging from 0.10 €/pig to a maximum of 18.73 €/pig according to EP-like lesion severity.
Conclusion: Based on abattoir inspections, this study indicates that *Mhyo* is highly prevalent among pig farms in Northern Italy. Accordingly, all batches investigated presented lung lesions compatible with EP, although its severity varied. In addition, we were able to estimate economic losses due to *Mhyo* under Italian conditions, showing that EP can cost 3.03 €/pig on average. Overall, this study highlights that EP is a relevant disease in Italy and the need for optimizing EP management practices, including *Mhyo* vaccination, and continued abattoir monitoring programmes.

Disclosure of Interest: None Declared

Keywords: None

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Development of multiplex PCR diagnostic assay for the detection of mycoplasmas in lung samples of slaughtered pigs under Thai swine field

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Introduction: Swine mycoplasmas are commonly acknowledgement for their potential to prompt porcine respiratory disease complex (PRDC) and an emergence or re-emergence of mycoplasmas-associated arthritis that have an impact on significant economic losses worldwide including Thailand. Nowadays, a molecular-based method has become powerful tool as diagnosing in both mycoplasmas research and clinical laboratories. In Thailand, little is reported regarding the development of molecular-based approaches for scrutinizing mycoplasmas-triggered PRDC in one step. Therefore, the present study emphasizes the development of a multiplex PCR diagnostic assay targeting 16S rRNA gene that has been proposed with used of differentiating between *Mycoplasma hyopneumoniae* and *Mycoplasma hyorhinis* and presumptive screening *Mycoplasma hyosynoviae* in lung samples of seventy slaughtered pigs.
Materials and Methods: Primers specific to the portion of 16S rRNA conserve region of mycoplasmas were designed using Bioedit V.4.5. Specificity and sensitivity of the assay were validated by *M. hyopneumoniae*, *M. hyorhinis* and *M. hyosynoviae* strains and other swine bacteria such as *Actinobacillus pleuropneumoniae, Escherichia coli, Haemophilus parasuis* and *Staphylococcus hyicus* and diluting tenfold of reference porcine mycoplasmas, respectively.
Results: Here we describe no cross-reactivity of non-target amplicons among several porcine bacteria except target mycoplasmas manifesting a specific band at 282 bp (*M. hyosynoviae*), 431 bp (*M. hyopneumoniae*) and 685 bp (*M. hyorhinis*) with the detection limit abruptly 10^4 copies number per ml. Under preliminary field trial, twenty-nine out of seventy lung samples (41.4%) showed *M. hyopneumoniae*-positive and sixty-three samples (90.0%) were positive to *M. hyorhinis* illustrating 431 bp and 685 bp, respectively. Additionally, mixed infection among mycoplasmas up to 31.4% (22/70) was observed, whereas no sample was positive to *M. hyosynoviae*. Nucleotide sequences of 15 target amplicons each of mycoplasma species demonstrated 99% homology to *M. hyopneumoniae* for instance GenBank accession no. Y00149 and *M. hyorhinis* including GenBank accession no. AF121890, respectively.
Conclusion: As a consequence, multiplex PCR assay in this study represents the valuable and rapid test with highly sensitive and specific to identify mycoplasmas-infected pigs within swine husbandry especially mycoplasmas-free herd, access strategies in view of medication planning and small and large-scale surveillance on Thai swine farms in further study.

Disclosure of Interest: None Declared

Keywords: Multiplex PCR , Porcine respiratory disease complex , Swine mycoplasmas

Bacteriology and Bacterial Diseases
MYCOPLASMA

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Guidelines for sample selection from replacement gilts and growing pigs for successful Mycoplasma hyopneumoniae culture and isolation

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Introduction: *Mycoplasma hyopneumoniae* (Mhp) has historically been challenging to culture from lung tissue samples of naturally infected swine. The most recent reported success rate for culture of Mhp was 8%. Many factors influence the success rate of culturing Mhp including the quality of the sample, presence of other bacteria in the sample and/or media, and laboratory growing conditions. Due to these obstacles, there was a need for work to determine best practices for successful culture of Mhp from field samples.
Materials and Methods: This work was performed in 4 continuous flow growing pig barns identified as Mhp clinically active, confirmed by observation of clinical signs and positive PCR on lung tissue. Prior to sample collection, a pre-screening protocol was designed and implemented. The first stage of the protocol consisted of a cross sectional sampling of ≥ 10 tagged pigs per age group (≥ 3 age groups) for laryngeal swab collection, 3-5 weeks after the onset of Mhp clinical signs. Groups with results ≥ 70% Mhp PCR positive were identified for further sampling. The second stage of the protocol consisted of selecting 2-4 Mhp PCR positive pigs with clinical signs including dry coughing and labored breathing, along with the lowest Mhp PCR Ct value in laryngeal swabs. Selected pigs were humanely euthanized and necropsied. Acute enzootic pneumonia like lesions were verified on gross lung tissue. The entire lung pluck (larynx to lungs) was collected and placed into clean bags. Bagged lungs were immediately placed on ice for transport to a freezer. Samples were placed in a -20ºC freezer. Frozen samples were transported and delivered ≤ 24 hours post-collection to the Mycoplasma Lab, University of Minnesota. It is hypothesized that freezing aids microorganism detachment from tissue resulting in higher bacterial recovery.
Results: Using the pre-screening protocol and methods described above, the authors have experienced a 100% culture and isolation success rate on the first collection attempt at all 4 barns sampled.
Conclusion: Under the conditions of this investigation, guidelines to improve the success rate of Mhp culture from lung tissue of naturally infected swine populations were generated. Utilization of the pre-screening protocol allowed for identification of the ideal specimen in terms of timing and degree of infection, which allowed the lab to procces reliable samples with enough microbial load for culture and isolation of Mhp. Increasing the Mhp library of diagnostic and research laboratories will allow for expansion of research in Mhp diagnostics, characterization, antibiotic resistance, gilt acclimation procedures, among other research areas.

Disclosure of Interest: None Declared

Keywords: Culture, Isolation, Mhyo

Poster Abstracts

Bacteriology and Bacterial Diseases
MYCOPLASMA

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COMPARATIVE FIELD STUDY: PORCILIS® M HYO ID ONCE AT FOUR WEEKS OF AGE VERSUS INTRAMUSCULAR DOUBLE DOSE MYCOPLASMA VACCINATION

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Introduction: The aim of this contemporary comparison trial was to test, under field conditions, an intradermal 1-shot *Mycoplasma hyopneumoniae* (Mhyo) vaccine versus an intramuscular double injection vaccination.
Materials and Methods: This study was carried out according to a randomized, sex stratified and semi-blinded design in a farrow-to-finish pig herd with clinical (serology based) Mhyo, Influenza and *Actinobacillus pleuropneumoniae* infection. Piglets from two consecutive 5-week-farrowing batches were allocated randomly, within litters, to one of two groups. The pigs in one group were vaccinated intramuscularly with Suvaxyn® M Hyo at 12 and 28 days of age. The other pigs were injected intradermally (IDAL®) with Porcilis® M Hyo ID Once at 28 days of age.
IPAL (Interprofession Porcine Auvergne-Limousin) slaughter data were collected, allowing the comparison of carcass data and calculation of average daily gain (ADG). Five slaughter checks were performed using Madec quantitative lung scoring method on 6 lobes (0-24), excluding the azygous lobe. Scar lesions and pleurisy were rated present/absent. ADG was tested by ANOVA, pneumonia scores were analyzed by the Kruskal-Wallis test and prevalence was analyzed by the Mantel-Haenszel test (batch adjusted) or the Fisher's exact test in case of insufficient data.

Results: Individual slaughter data from 1555 pigs was collected and a total of 1089 lungs were checked at the abattoir (respectively 66% and 47% of each included batch). ADG (668 gr/day for Porcilis® and 662 gr/day for Suvaxyn®) and average lesion scores (1.8 for Porcilis® and 2.1 for Suvaxyn®) were not statistically different between groups, as well as pneumonia and pleurisy prevalence. Slaughter data established that there was no statistical difference between groups for muscle rate and carcass weight distribution.
After a year of vaccination of 100% of the animals, the average results of slaughter checks improved significantly compared to the previous average results (pigs vaccinated with two intramuscular injections).

Conclusion: In this study, no statistically significant differences were found between the group of animals vaccinated intradermally with a single dose Mhyo vaccine and a group vaccinated intramuscularly with a double Mhyo dose protocol. Despite, the farmer was pleased with the working comfort and overall quality of the vaccination procedure when using IDAL.
After one year of intradermal vaccination of 100% of piglets with Porcilis® M Hyo ID Once, average lung lesion score improved to 0.9 and no more collective antibiotic treatments in feed were administered in the fattening period (weaning-to-sale loss rate below the 3%).

Disclosure of Interest: None Declared

Keywords: Mycoplasma hyopneumoniae, Porcilis® M Hyo ID Once, vaccination

Bacteriology and Bacterial Diseases
MYCOPLASMA

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Effect of vaccination against Mycoplasma hyopneumoniae of breeding pigs pre-farrowing on colonization rates of piglets at weaning

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Introduction: *Mycoplasma hyopneumoniae* is one of the primary pathogens involved in the Porcine Respiratory Disease Complex. Breeding sows are responsible for maintaining *M. hyopneumoniae* infections within the herd and young sows are more likely to transmit the pathogen to their piglets compared to older sows. Additionally, piglet colonization at weaning has been suggested as a predictor of clinical disease and lung lesions at slaughter. The aim of this study was to investigate whether vaccination of sows against *M. hyopneumoniae* at the end of gestation can reduce the colonization rate of piglets at weaning.
Materials and Methods: Two farrow-to-finish herds were selected; herd A (400 sows) and herd B (600 sows). From 6 consecutive farrowing groups of sows in each herd, 3 groups were vaccinated against *M. hyopneumoniae* and 3 remained non-vaccinated. Vaccination of the groups was applied in an alternating way. In vaccinated groups, all gilts and sows received twice a commercial bacterin (Ingelvac MycoFLEX®) at 6 and 3 weeks before the expected farrowing date. Then, from each vaccinated or non-vaccinated group of sows within each herd, 5 primiparous sows together with their litters were selected. Laryngeal swabs were collected from each primiparous sow within 24 hours after parturition, and additionally at the day of weaning from all piglets belonging to the selected primiparous sows. Collected swabs were tested for the presence of *M. hyopneumoniae* using a nested PCR. The vaccinated groups were compared with the non-vaccinated ones using logistic regression.
Results: In herd A, 10% of each of the vaccinated and non-vaccinated primiparous sows were positive for *M. hyopneumoniae* (P=0.998). In this herd, 0.86% and 1.60% of the piglets born from the vaccinated and non-vaccinated primiparous sows, respectively, were positive for *M. hyopneumoniae* at weaning (P=0.584). In herd B, 0% of the vaccinated and 20% of the non-vaccinated primiparous sows were positive for *M. hyopneumoniae* (P=0.598). In this herd, 1.06% and 3.12% of the piglets born from the vaccinated and non-vaccinated primiparous sows, respectively, were positive for *M. hyopneumoniae* at weaning (P=0.176). The risk for the piglets of the non-vaccinated primiparous sows to be positive for *M. hyopneumoniae* in herd A was 1.96, while in herd B was 2.95 times higher when compared to that of the piglets of the vaccinated primiparous sows.
Conclusion: In both herds, piglets originating from the vaccinated primiparous sows had 2 to 3 times lower risk of being colonized with *M. hyopneumoniae* at weaning. More litters will have to be investigated, since statistics were not able to demonstrate a clear benefit, likely due to the low number of colonized piglets.

Disclosure of Interest: None Declared

Keywords: Colonization, Mycoplasma hyopneumoniae, Vaccination

Bacteriology and Bacterial Diseases
MYCOPLASMA

PO-PF3-028

Field application of Mycoplasma hyopneumoniae molecular characterization and analysis tools

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Introduction: Several tools have become readily available for molecular characterization and analysis of *Mycoplasma hyopneumoniae* (Mhp). With increasing interest in control and elimination of Mhp, there was a need to better understand the epidemiology in the field. Questions of interest included evidence of Infection Chain™ versus lateral transmission in the growing pig and identification of variant(s) within sow herds prior to Mhp herd elimination programs to attempt to determine variant origin in the event of identification of Mhp post-elimination.
Materials and Methods: In order to begin documentation of Mhp infection/exposure between production stages, samples from known Mhp positive gilts, sows, nursery pigs and finishing pigs in the same flow were targeted for laryngeal swab sampling. Samples from surrounding sites, with no flow similarity to the target sites, were requested to be included in the project. Ten pigs were sampled per production stage, targeting clinical signs associated with Mhp(dry coughing and labored breathing). Pigs recently treated with Mhp sensitive antibiotics were not sampled. In order to document lateral spread, a minimum of 20 flows were planned to be targeted with at least 100 samples analyzed. Mhp PCR positive laryngeal swab samples with a Ct≤32 were sent to the UM-VDL for P146 full sequence anlaysis and to the Mycoplasma Lab, UM for MLVA analysis. P146 full sequences and MLVA typing for each variant were uploaded into the Disease BioPortal (CADMS, UC-Davis), along with the corresponding site information for temporal-spatial genomic analysis.
Results: To date molecular characterization has been successfully completed on 6 flows, including 39 sites and 51 sequence and typing events. The Disease BioPortal temporal-spatio-genomic visualizer has been utilized for analysis of Mhp P146 full sequences and MLVA typing. The authors have not identified lateral transmission of Mhp, but rather transmission has appeared to occur within the Infection Chain™/vertical within flow, as suggested by the similarity of sequences and types of variants from vertically related sites. A database has been created for variants from each site.
Conclusion: Tools for molecular characterization and analysis of Mhp are available for use in the field. These tools, including P146 full sequences, MLVA typing and the Disease BioPortal allow for a better understanding of the transmission of Mhp in the field. Under the conditions of this project, transmission of Mhp appears to occur within the Infection Chain™. Lateral transmission of Mhp has not been documented in this project to date. A database has been created tohelp determine Mhp variant orgin in the event of Mhp identification within flows post-elimination.

Disclosure of Interest: None Declared

Keywords: Characteristics, Infection Chain, Mhyo

Bacteriology and Bacterial Diseases
MYCOPLASMA

PO-PF3-120

Monitoring of respiratory pathogens in multi-sourced gilt developing units

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Introduction: Respiratory diseases are highly prevalent in swine populations, including reproductive herds. Currently, there is lack of information regarding the co-infection dynamics of *M. hyopneumoniae* (*Mhp*), Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), and Influenza A Virus (IAV) detection and correlation across gilt developing units (GDUs). Therefore, the objective of the study was to monitor respiratory pathogens among multi-sourced GDUs.
Materials and Methods: Four GDUs positive for *Mhp*, PRRSV, and IAV were used for this study. Gilts were vaccinated for *Mhp* and PRRSV and were considered stable for the main respiratory pathogens. Gilt groups were longitudinally monitored by collecting oral fluid (OF) samples from pens per age group (8, 12, 16, 20, 24, 28, and 32 weeks) every 4 weeks for 11 sampling events. Ropes were shared between two pens that housed 20 gilts of the same age. Each OF sample was tested for *Mhp*, IAV, and PRRSV by qPCR. The correlation of gilt age per GDU with the percentage of OF positive for each pathogen was calculated. Laryngeal swabs (LS) were collected from 30 gilts per age group per GDU at the first sampling event. LS were pooled by age in groups of 5, tested for *Mhp* by qPCR, and an average Ct value was determined for each age group per GDU.
Results: In one GDU, age had a significantly negative correlation with the percentage of OF positive for PRRSV (p=0.00). There was no significant correlation between age and the percentage of positive IAV and *Mhp* OF (p=0.2). In the remainder 3 GDUs, the percentage of OF positive for PRRSV and IAV had a significantly negative correlation with age compared to *Mhp*, which had a significantly positive correlation (p=0.00). In all GDUs, LS detected *Mhp* approximately 4 weeks earlier than OF and resulted in a higher percentage of gilts positive. The initial detection of *Mhp* by age varied in all GDUs. Across all GDUs, 100% of 8 and 12 week old sampled gilts were *Mhp* negative and all 32 week old gilts were positive.
Conclusion: Three of the 4 GDUs showed similar patterns of detection for *Mhp*, IAV, and PRRSV in OF. The percentage of positive oral fluids for IAV and PRRSV decreased from 8-32 weeks of age, while the percentage of positive oral fluids for *Mhp* increased with age. Although two GDUs were sourced from the same site, patterns of infection of IAV and *Mhp* differed. When comparing LS with OF across all GDUs, LS had a higher sensitivity for *Mhp* detection. Detection patterns for IAV, PRRSV, and *Mhp* differed by sample type, age, and GDU. Results from this study suggest that the age of testing and types of samples should be different depending on the pathogen of interest.

Disclosure of Interest: None Declared

Keywords: Multi-sourced GDUs, Mycoplasma hyopneumoniae, PRDC

Poster Abstracts

Bacteriology and Bacterial Diseases
OTHERS

PO-PF3-007

Enteric carriage of a septicaemia-associated Klebsiella pneumoniae strain by pigs in England

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Introduction: Between 2011 and 2014, outbreaks of septicaemia due to *Klebsiella pneumoniae* subspecies *pneumoniae* (*Kpp*) were diagnosed on sixteen commercial English pig farms. Prior to 2011 *Kpp* was seen rarely as a cause of mastitis and sporadic infections in pigs. The outbreak presentation has a seasonal occurrence (May to September) and occurs predominantly in outdoor pigs aged ten days old to weaning. Molecular characterisation of outbreak *Kpp* isolates showed that by multi locus sequencing all were sequence type 25 (ST25) with a unique combination of a 4.3kb plasmid and *rmpA* virulence gene. During 2015, enteric carriage of the outbreak *Kpp* strain by pigs was investigated by sampling diagnostic submissions to APHA. *Kpp* ST25 has not been reported associated with human infection in the UK.
Materials and Methods: Active surveillance for large intestinal or faecal carriage of *Kpp* in porcine diagnostic submissions to APHA was undertaken over the twelve months of 2015, regardless of age (excluding foetuses) and disease presentation. The first 100 samples were inoculated onto HiCrome *Klebsiella* selective agar (Oxoid), Simmons citrate & 1% inositol, SBA, MacConkey, MacConkey with added ampicillin (6mg/l) and Todd-Hewitt broth with added ampicillin (6mg/l) to assess the best culture method. Subsequently only Simmons citrate & 1% inositol was used. All *Kpp* isolates were then tested using a PCR to detect the presence of rmpA and the 4.3kb plasmid.
Results: The first 100 samples cultured resulted in the isolation of 14 *Kpp* isolates. Twelve of these 14 isolates were via Simmons citrate & 1% inositol media with one isolation of *Kpp* for each of Todd-Hewitt broth with added ampicillin (6mg/l), HiCrome *Klebsiella* selective agar and MacConkey agar. *Kpp* isolates were obtained from 58 (17%) of 336 faecal/intestinal samples. Ten of 47 *Kpp* isolates characterised so far (21%) are genotypically similar to the outbreak strain *Kpp* ST25. Of these, enteric carriage of ST25 was 90% in pigs with *Kpp* septicaemia and 0.4% in pigs not affected by *Kpp* septicaemia.
Conclusion: Pigs affected with *Kpp* ST25 septicaemia had high enteric carriage of the outbreak strain confirming potential for faecal spread of the disease-associated *Kpp* strain. Carriage of *Kpp* ST25 in pigs affected by diseases other than *Kpp* septicaemia was very low but finding *Kpp* ST25 in these pigs is significant as evidence that the strain can be present in herds in which disease is not apparent. There was no apparent seasonal trend in *Kpp* or *Kpp* ST25 isolation. The epidemiology of this emerging disease is not fully understood but this study adds to the growing evidence base and emphasises that implementing good biosecurity is likely to help limit spread of *Kpp* ST25.

Disclosure of Interest: None Declared

Keywords: klebsiella , Pig feces, septicaemia

Bacteriology and Bacterial Diseases
OTHERS

PO-PT2-002

Clostridium difficile in Irish pigs – a preliminary study.

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Introduction: *Clostridium difficile* is an enteropathogen of humans and animals. It is a major cause of porcine neonatal diarrhoea in the US and Europe but the role of *C. difficile* in neonatal diarrhoea in Irish pigs is unknown and typhlocolitis due to *C. difficile* has not been confirmed. In addition, community-acquired *C. difficile* is an important emerging trend in human *C. difficile*-associated disease (CDAD) and potential sources, including farm animals, are now of considerable interest. The objectives of this study were to investigate *C. difficile* pathology and epidemiology in pigs referred to the Central Veterinary Research Laboratory (CVRL), Backweston and to compare strains identified with those identified in human CDAD.
Materials and Methods: Colonic contents sampled from pigs referred to the CVRL for necropsy with (n=73) and without (n=74) a history of diarrhoea were subject to toxin ELISA. PCR ribotyping was done on 49 *C. difficile* isolates. Where suitable samples were available, histopathology was performed on representative areas along the length of the small intestine, caecum and colon of piglets presenting with diarrhoea (n=62).
Results: *C. difficile* toxins were detected in 77/147 samples from all 22 farms tested, in animals with (n=47, 64%) and without (n=30, 40%) diarrhoea. Nine piglets had histopathological lesions in caecum and colon consistent with CDAD and a further 33 had mild, inconclusive histopathological changes. All piglets with diarrhoea also had lesions attributable to other pathogens in the small intestine and the small intestinal lesions were generally more severe than the colonic lesions. To date five ribotypes of *C. difficile* have been identified: R 078 (32%), R 014/020 (27%), R 110 (25%), R 017 (14%) and R 011 (2%).
Conclusion: This study presents the first findings of colitis associated with *C. difficile* in Irish pigs. Piglets with diarrhoea and toxin detected had a spectrum of histopathological changes. Lesions attributable to CDAD were never the sole enteric lesions present in these pigs, suggesting that *C. difficile* does have a role in porcine neonatal diarrhoea in Ireland as part of a multifactorial problem. The results also suggest that, similar to findings in other countries, porcine colonisation with toxigenic *C. difficile* is not uncommon in Ireland, even in the absence of clinical signs. In this first report of PCR ribotyping in Irish pigs, the diversity of ribotypes, and ribotypes identified, reflect those associated with human CDAD in Ireland. Further research, including whole genome sequencing, is required to investigate the relationhip between strains in pigs and humans.

Disclosure of Interest: None Declared

Keywords: Clostridium difficile, Colitis, Colonisation

Bacteriology and Bacterial Diseases
OTHERS

PO-PF3-018

Clinically relevant antibiotic resistance in Escherichia coli from urinary tract infections in sows.

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Introduction: *Escherichia coli* is the most frequently isolated bacteria from urinary tract infections (UTI) in sows. Antibiotic treatment is commonly used for treatment of peri-parturient diseases of sows including UTI. However, information on antibiotic resistance in *E. coli* isolated from sow urine is limited, and therefore it has been difficult to provide guidelines for selection of antibiotics in relation to treatment of UTI.
The aim of this study was to investigate antibiotic resistance patterns of *E. coli* isolated from the urinary tract of sows.
Materials and Methods: Urine samples were randomly collected from 179 sows at a Danish slaughterhouse during 2014. The samples were taken by cystocentesis immediately after removal of the bladder at the slaughter line. Urine culture was performed and pure bacterial cultures with ≥ 1000 CFU/ml urine was considered indicative of cystitis.
Amoxicillin (amox), amoxicillin+clavulanic acid (amox+clav) and sulfamethoxazole+trimethoprim (sulfa+tmp) were considered as clinical relevant antibiotics for treatment of UTI in sows. Doxycycline was also included since doxycycline has been widely used in the Danish pig production. The 3rd generation cephalosporines has not been used in Danish pig production since 2010, but was included as indicator of ESBL producing E. coli.
Antibiotic susceptibility testing was performed with the Sensititre system. Based on clinical breakpoints the *E. coli* was divided into susceptible, intermediate and resistant isolates.
Results: A total of 55 bacteria cultures were isolated in pure culture with bacterial count ≥ 1000 CFU/ml urine, and 44 of those were *E. coli*. Susceptibility testing was performed on 40 *E. coli* isolates from different herds.
While 29 (73 %) of were resistant to amox only 3 (8 %) was resistant to combined amox+clav and 4 (10 %) was intermediate. For sulfa+tmp 23 (58 %) of isolates were categorized as resistant and for doxycycline 9 (23 %) of the isolates were resistant and 14 (35 %) were intermediate. Resistance to 3rd generation cephalosporines, which is indicative of ESBL producing *E. coli*, was observed in 3 (8 %) isolates.
Conclusion: Widespread antibiotic resistance against clinically relevant antibiotic compounds available for treatment of *E. coli* from UTI in sows was observed in this study. Therefore antibiotic susceptibility testing is highly relevant before treatment of UTI.
Amox+clav was recently approved for use in the Danish pig production, and resistance to this combination is at the moment at a lower level than the other therapeutic alternatives.
ESBL-producing *E. coli* are at a relatively low level in in UTI isolates from Danish sows.

Disclosure of Interest: None Declared

Keywords: antibiotic resistance, sows, urinary tract infection

Bacteriology and Bacterial Diseases
OTHERS

PO-PF3-017

Diagnostic performance of bacteriological culture of urine samples from culled sows, using histopathologically demonstrated cystitis as gold standard

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Introduction: Urinary tract infections (UTI) in sows have been investigated occasionally during the last 30 years, but prevalence of UTI in Danish sows in Denmark is currently unknown. Furthermore, the definition of cystitis in sows has been discussed, and histopathology has been suggested as gold standard, but the histopathological criteria have not been consistent. Therefore the primary objective of this study was to develop histopathological criteria for diagnosis of cystitis in sows.
The aim of this study was, to determine the prevalence of histologically verified cystitis in culled sows and to compare the histopathological findings with bacterial counts in related urinary samples.
Materials and Methods: A total of 175 sow bladders from 105 herds were collected from a Danish slaughterhouse. Urine was taken aseptically by cystocentesis of the removed bladder within 4 hours after removal. The urine was cultured on standard KA blood agar aerobically for 18-24 hours and the results was given on a semi-quantitative scale ranging from 0 to 1.000.000 colony forming units (CFU) pr. mL urine. CFU counts ≥1000, and with a pure culture, were considered bacteriuria.
From each bladder body a 2x3 cm tissue sample was fixed in 10% formalin, processed for histopathology, and stained with hematoxylin and eosin. Each sample was microscopically evaluated blinded by the same person and the following findings were recorded: number of mononuclear cells, number of neutrophils, presence of hyperemia and lymphocytic foci. The cut-off for chronic cystitis was presence of ≥ 40 mononuclear cells pr. high-power field (HPF). For acute cystitis the cut-offs were ≥ 40 mononuclear cells pr. HPF and hyperemia.
Results: The results showed that 55 (31 %) of the sows had bacteriuria. Histopathology revealed that 75 (43%) had cystitis with chronic cystitis in 12 (7 %) and acute cystitis in 63 (36 %).
Lymphocytic foci were found to be an occasional finding, as these were not associated with other pathological findings. Neutrophils were seen in both chronic and acute samples.
The diagnostic performance for urine culture, when compared to histopathological cystitis, was: sensitivity = 0.83 [0.70; 0.91] and specificity = 0.78 [0.69; 0.85].
Conclusion: In a sample of 175 Danish sows bacteriuria was demonstrated in 31%. Histopathological cystitis was present in 43 % of the samples. In 83 % of the cases of histopathological cystitis, bacteriuria was also present. Histopathological cystitis was defined as presence of ≥ 40 mononuclear cells pr. HPF and ≥ 40 mononuclear cells pr. HPF plus hyperemia for chronic and acute cystitis respectively.

Disclosure of Interest: None Declared

Keywords: diagnosis, sows, urinary tract infection

Poster Abstracts

Bacteriology and Bacterial Diseases
OTHERS

PO-PF3-202

Cure rate of spontaneous clinical swine respiratory disease with a single dose of marbofloxacin 16% in fattening swines within an integration system

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Introduction: The swine respiratory disease (SRD) caused by bacteria is enzootic character and spread widely in Brazil and it is present in most of the fattening units.
The SRD represent a high economic cost, causing mortality, developmental delay, lack of uniformity of lots, spending on drugs and condemnation of carcasses.
The prudent use of antibiotics is becoming increasingly necessary in animal production, with it, the use of marbofloxacin 16% (Forcyl®), an antibiotic concentration dependent with action directed to Gram-negative bacteria combined with a new concept (SISAAB - Single Injection Short Action Antibiotic), has taken power and space. This concept consists in using a bactericidal antibiotic in high concentration, fast action with a minimum exposure time, controlling infection and favoring the natural immunity of the animal.
This study aimed to evaluate the cure rate of the spontaneous clinical respiratory disease using a 16% marbofloxacin (Forcyl®) in fattening swine in an integration system.
Materials and Methods: 12,986 animals were used, with average weight at 24 kg at the time of housing in eight fattening units located in the state of Minas Gerais, Brazil. According to the routine of integrating company, we considered the following criteria for SRD in the first 120 days of accommodation: tachypnea, cough, sharp, appetite loss and ability to remain on station. Animals that has two or more of those characters were classified as patients and then treated with a single application of IM marbofloxacin (Forcyl®) 8mg / kg, equivalent to 1ml / 20ml live weight and followed the clinical course of the animal. The occurrences of diseases, treatments and deaths were recorded in monitoring reports, thus we evaluated the clinical cure rate with treatment used. Besides that, all management standards, cleaning, disinfection, food and other medications followed the standards set by the integrator.
Results: 4,126 animals were treated in a total population of 12,986 swines. The total number of deaths from SRD was 86 animals. We obtained the following results; 0.66% of deaths when we consider the complete population in the units, 2.08% of SRD deaths when we consider the number of treated animals and cure rate was 97.92%.
Conclusion: In conclusion, the marbofloxacin 16% (Forcyl®) using a single intramuscular injection showed to be safe and effective in the treatment of spontaneous clinical SRD in animals inside of a integration system. The cure rate of 98 % suggests minimization of economic losses resulting from respiratory diseases.

Disclosure of Interest: None Declared

Keywords: marbofloxacin 16%, SISAAB , swine respiratory diseases

Bacteriology and Bacterial Diseases
OTHERS

PO-PF3-043

Prevalence Mycobacterium species and its rifampin and isoniazid resistance genes in Slaughter Pigs in Korea.

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Introduction: Tuberculosis (TB) is an infectious, granulomatous disease caused by acid-fast bacilli of the genus *Mycobacterium*. The disease affects practically all species of vertebrates. Although mammalian tuberculosis has been nearly controlled in many developed countries, it is still a serious problem in humans and domestic animals including pigs. Previous studies have shown that pigs are among the possible sources of mycobacterial infections to humans and animals. Rifampin (RIF) and isoniazid (INH) are crucial elements of the standard treatment regimen of human tuberculosis, and resistance to these drugs requires extension of therapy. The aim of this study is to identify different *Mycobacterium* species and its RIF and INH resistance associated with swine mycobacteriosis in pigs that could represent a potential public health concern in Korea.
Materials and Methods: Two hundred and ten pig lymph nodes were obtained from slaughter house in Jeonbuk province, Korea. The DNA was extracted using DNA extraction kit (DNeasy blood and tissue kit, Qiagen) according to the manufacturer's instructions. The isolated DNA was used as template in the POBGEN™ Mycobacterium (MTC/M. bovis) screening kit (Postbio, Inc., Korea). Anyplex MTB/NTM testing (Seegene, Korea) used 5 µl of extract which was added to a 15-µl master mix containing 10 µl 2× Anyplex PCR master mix, 3 µl methoxypsoralen (8-MOP), and 2 µl 10× *M. tuberculosis*/NTM oligonucleotide mix.
Results: Seventy eight of 210 lymph nodes (37.14%) were positive for MTC and NTM. Out of 78 samples, 42 samples (53.84%) were positive for MTC, whereas 36 samples (46.15%) were positive for NTM by real-time PCR. Among 78 positive samples to *M.* spp, 37 (49.33%) were confirmed to have drug-resistant gene to RIF and/or INH. Among 31 cases identified as *M. bovis*, there were identified as 10 having RIF-resistant gene, 3 having INH-resistant gene, and 3 cases having both drug-resistant genes. Eleven cases with *M. tuberculosis* were classified as 1 with RIF-resistant gene, 2 with INH-resistant gene and 1 with both drug-resistant genes. It is the first report on the demonstration of anti-TB drug-resistant genes against *Mycobacterium* spp materials originated from pigs in Korea.
Conclusion: Consequently, the study obtained was strongly suggested that there is mostly likely to have the infective cycle of four *M.*. spp including *M. bovis*, *M. tuberculosis, M. avium* and *M. porcinum* between human beings and Korean pigs. That is the reason that the further study of TB should be designed to more extensive and systemic work focusing the interspecies transmission of TB among the susceptable hosts.

Disclosure of Interest: None Declared

Keywords: Mycobacterium, Prevalence, Slaughter Pigs

Bacteriology and Bacterial Diseases
OTHERS

PO-PF3-004

Oral microbiota in piglets at the nursery phase

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Introduction: The oral cavity of animals harbors an extensive microflora, including indigenous commensals and other microorganisms. Oral microbiota may play an important role in the protection of the host against pathogenic infection through the inhibition of colonization of pathogens or growth competition. Especially, the composition of oral microbiota of piglets at the nursery phase, at the critical time to establish a normal flora for life-time, would be drastically altered by external event, such as weaning, feeding, dietary change, and eventually influenced on the growth performance. Thus, the aim of the present study was performed to analyze oral microbiota of piglets from newborn to early stage of nursery in order to extend our understanding of the composition and alternation of oral microbiota.
Materials and Methods: One hundred samples were collected by vigorously scraping oral cavity with cotton swabs from clinically healthy piglets, assigned into 5 different groups; newborn, suckling only, suckling (with feeding), 6 weeks and 8 weeks of age. Ten swabs in the designated group were pooled into a single sample. Bacterial genomic DNA was directly extracted from the pooled samples and subjected to emulsion-based PCR to amplify 16S rRNA gene. Next Generation Sequencing (NGS) was applied for sequencing the amplified product with a Roche 454 GS-FLX plus (Macrogen, Korea). Taxonomy of 16S rRNA of all sequence reads was assigned by using Silva rRNA and NCBI taxonomy database.
Results: NGS analysis results showed 111,389 of overall number of reads and 51,939,792 bases in the total length for 10 samples. At the level of phylum, both Firmcutes and Proteobacteria were dominant microflora in the range of 80~ 90 % of all number of reads. However, Streptococcaceae is main microflora, followed by Moraxcellaceae, Lactobacillaceae, Neisseriacease, and Pasteurellaceae, in all groups, except newborn piglets. Surprisingly, some major swine pathogens, such as Mollicutes, Pasteurella, Haemophilus, Actinobacillus and Erysipelothrix, were found as early as 2 weeks of age in piglets. Although these pathogens are opportunistic bacteria in the upper respiratory tract, it should be considered as a risk factor for swine health and management.
Conclusion: Oral microbiota of animals is constantly altered, dependent on the conditions encountered during growth phase, diet change, and environment. Thus, further studies should be needed to expand our knowledge on the composition and alteration of oral microbiota of pigs at differnt conditions, which may be eventually helpful to improve a herd performance.

Disclosure of Interest: None Declared

Keywords: NGS, oral microbiota, piglet

Bacteriology and Bacterial Diseases
OTHERS

PO-PF3-059

First isolation of Brucella suis biovar 2 in wild boar in Finland

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Introduction: Porcine brucellosis, an infectious disease caused by *Brucella suis* biovar 1, 2, or 3, is a zoonotic disease of public health and economic concern. The infection generally manifests itself as a reproductive disease potentially leading to abortion in sows and infertility in sows and boars. In Europe, the most common agent of swine brucellosis is *B. suis* biovar 2 which is endemic in European wild boar and hare populations. These wild animals may spread the infection to domestic pigs causing significant losses in the pig industry.
The aim of this study was to determine the presence of *B. suis* bacteria in the wild boar population in Finland by analyzing the samples sent to the Finnish Food Safety Authority Evira as a part of the national monitoring program for African Swine Fever in wild boar.
Materials and Methods: Specimens sent by hunters contained blood and/or organ samples (spleen, kidney and uterus or testicle) from altogether 133 animals, mainly from the south and south-eastern part of the country. Blood samples were tested for brucella antibodies by serological methods. The organs from antibody test- positive animals were cultured for brucella. Organ samples were also cultured from the animals which could not be tested for brucella antibodies because of lack or poor quality of blood sample.
Blood samples were tested by using three serological methods (Rose Bengal, Complement Fixation and ELISA). For bacteriological examination organ samples were cultivated on selective Farrell's medium and blood agar. Cultures were incubated at 37°C and followed up for 10 days. Presumptive identification was based on Stamp-staining, biochemical tests and PCR assay targeting the IS711. Further identification of PCR positive strains was done at EURL for Brucellosis in France.
Results: Blood samples from five out of 112 (4.5%) wild boars were antibody positive by all three methods. Four of them originated from male and one from female animals. Organ samples from 37 animals, including the five antibody positive animals, were cultured. Two out of 37 animals were positive by culture. Both animals were males hunted in the south-eastern part of Finland. All culture positive animals were also antibody positive. Both brucella isolates were identified as *B. suis* biovar 2. Organ samples from other three antibody positive animals were negative by culture. This may be due to the poor quality of organ samples.
Conclusion: The result of this study shows for the first time that *B. suis* biovar 2 is also present in wild boar population in Finland.

Disclosure of Interest: None Declared

Keywords: Brucella suis, wild boar

Poster Abstracts

Bacteriology and Bacterial Diseases
OTHERS

PO-PF3-104

Development of a Pulsed-Field Gel Electrophoresis (PFGE) method for molecular typing of Trueperella pyogenes isolates.

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Introduction: *Trueperella pyogenes* is an opportunistic pathogen related to a high spectrum of miscellaneous suppurative infections in pigs, including metritis, udder lesions, abscesses, pneumonia, arthritis, endocarditis, lymphadenitis and osteomyelitis. However, little is known regarding genetic diversity among isolates. In this work, the development of a PFGE assay to analyse clinical isolates of this pathogen is evaluated.
Materials and Methods: A total of 32 *T. pyogenes* isolates obtained from different organic tissues (tonsils, lymph nodes, lungs, liver and spleen) of 8 free-range pigs totally condemned due to the presence of generalised pyogranulomatous lesions were analysed. The DNA of these isolates was digested with 6 different restriction enzymes, including *Sfi*I, *Sma*I, *Bsp*120I, *Xba*I, *Xho*I and *Bcu*I. DNA fragments were resolved on a 1% agarose gel at 14 °C with 0.5X TBE buffer using a CHEF-DR® III system. All restriction enzymes were evaluated in a range of 10-20 U during a 4 h-overnight digestion period. When PFGE was performed, the following parameters were used: running time, 24 h; voltage gradient, 6 V; included angel, 120ºC; initial pulse time: 0.1 s; final pulse time: 10 s. The PFGE patterns were examined visually and the genetic relationship among isolates was evaluated following the Tenover criteria.
Results: Well-resolved macrorestriction profiles showing 15 to 18 DNA fragments were obtained when *T. pyogenes* DNA was digested with *Bcu*I enzyme. When this enzyme was used, all isolates were successfully characterized by PFGE after genomic DNA digestion, being identified 6 different pulsotypes (A, B, B1, C, D, D1).
Conclusion: PFGE using the enzyme *Bcu*I was a reproducible method for characterising *T. pyogenes* isolates and may be useful in molecular epidemiological studies of this pathogen.

Disclosure of Interest: None Declared

Keywords: PFGE, T. pyogenes

Bacteriology and Bacterial Diseases
OTHERS

PO-PF3-070

Infection Dynamics of Yersinia in a Pig Population

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Introduction: *Yersinia enterocolitica* infection in pigs has several implications as an enteropathogen of pigs and humans, and as a cause of false-positive serological reactions (FPSR) in tests for brucellosis (international trade implications). Swine are the primary reservoir of pathogenic *Yersinia enterocolitica*, although environmental survival may also be important. Few studies focused on the dynamics of infection at farm level. In pigs, *Y. enterocolitica* infection spreads by the feco-oral route and it is widely distributed across EU pig farms.The goal of this study was to evaluate infection dynamics in commercial pig production to support optimization of control methods.
Materials and Methods: A continuous flow 6000 pig growing farm in Germany was studied. Routine historical surveillance in had showed a variable prevalence of antibodies against *Y. enterocolitica*. Serum samples for a longitudinal study of 10 pigs within the herd were taken from 10 weeks up to 26 weeks of age. Antibodies against pathogenic Yersinias were tested at the Innovative Veterinary Lab of Hannover using an Elisa test (PIGTYPE YopScreen (LDL, Leipzig)).
Yersinia shedding:
1. Sock samples of pooled faeces: 10 socks were taken from the environment across the farm, sock was used to collected a poled floor sample from each of the 10 mixed age buildings.
2. Individual fecal samples: A cross sectional study taking individual fecal samples was performed in 4 groups of 20 pigs ranging from 16 weeks up to 21 weeks of age to evaluate bacterial shedding.
Isolation from socks and fecal samples was attempted using the cold enrichment method at the University of Veterinary Medicine of Hannover lab.
Results: No antibodies against Yersinia were detected at nursery and before 18 weeks of age. Seconversion started at 18 weeks of age (10% prevalence), increasing up to 25 weeks (100% of prevalence). All the sock samples were negative at culture and the results from fecal samples are ongoing (results will be presented at the conference).
Conclusion: Control of *Yersinia* infection in pigs is not easy as several factors can contribute to transmission at herd level. In this study, piglets received from the breeding farm were negative at arrival, no infection was observed at nursery, and the spread of the infection was delayed until 16 weeks of age. Environmental faeces samples were not useful to detect the shedding of the bacteria as reported in previous studies and bacterial isolation was difficult because of low levels of excretion. Defining the farm specific infection dynamics is key to establishing effective pig-flow and environmental disinfection-based control measures for this significant bacterium.

Disclosure of Interest: None Declared

Keywords: control strategies, Infection dynamics, Yersinia

Bacteriology and Bacterial Diseases
OTHERS

PO-PF3-020

Isolation of Rhodococcus equi from tuberculous-like lesions found in the submaxillary glands of pigs at slaughter in Ireland

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Introduction: REGULATION (EC) No 854/2004 OF THE EUROPEAN PARLIAMENT AND COUNCIL lays down specific rules for the organisation of official controls on products of animal origin intended for human consumption. In the Republic of Ireland (ROI) these controls are conducted by the official veterinarian, assisted by approved veterinarians.
The national pig herd in ROI is predominantly indoors (>98%), with limited or no access to animal vectors of Tuberculosis (TB) (deer & badger). There are no recent records of TB being isolated from slaughter pigs in ROI.
Inspection of the head, throat, mouth, pharyngeal area and tongue is visual. Incision and examination of the submaxillary lymph nodes compliments visual inspection.
Materials and Methods: A major ROI slaughterhouse has a monthly throughput of approx. 75,000 slaughter pigs. During a 5-month period in 2015, fifteen (15) tuberculous-like lesions were identified at post mortem in submaxillary lymph nodes. These were sent for culture to determine presence of TB. Eight individual farms were associated with these 15 lesions.
Farm feeding systems for slaughter pigs were reviewed on the eight source farms.
An additional twelve similar-type lesions were identified in a subsequent 4-month period. These were sent to a specialised laboratory for more detailed culture and identification purposes to determine the virulence of any *R equi isolates*.
Results: Tuberculous-like lesions were found in 0.02% of monthly slaughtered pigs at this processing facility.
Eight of the initial fifteen (53%) samples submitted revealed cultures of *R equi.* No evidence of *M.avium, M.bovis* or *M.tuberculosis* or any other bacterium was found in any of the samples.
Carcases from tuberculous-like lesions at slaughter were previously eliminated from the food chain at the Food Business Operator's (FBO's) expense. As a result of these *R equi* cultures, only head meat is now discarded with the residual carcase deemed fit for human consumption.
Seven of the eight source farms supplying the affected pigs had dry feeding systems.
Conclusion: *Rhodococcus Equi* is an environmental pathogen, present in soil and dust. Dust is omni-present on pig farms, especially those with either dry-feeding or litter bedding systems. Dust and its associated pathogens are clearly linked to these lesions at slaughter. *R equi* poses a significant threat to the health of both farm operatives and pigs.
R.equi has long been discovered in immune-compromised human patients, particularly those afflicted with HIV.
Modern pig viruses (PRRS & PCV2) have a considerable immunosuppressive effect. *R.equi* causes a cough in all species affected. *R.equi* may play a minor role in Porcine Respiratory Disease Complex (PRDC).

Disclosure of Interest: None Declared

Keywords: Rhodococcus equi, Slaughterhouse, Tuberculosis

Bacteriology and Bacterial Diseases
OTHERS

PO-PF3-021

Anti-SpaA Erysipelas-specific humoral immunity conferred by PARVORUVAX® in pigs

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Introduction: PARVORUVAX (Merial, Lyon, France) is a combined adjuvanted vaccine against porcine parvovirus and E*rysipelothrix rhusiopathiae.* Spa is the main surface protein of the bacillus and is classified into three molecular species including SpaA. SpaA is present in essentially all virulent strains for swine species and has been shown to elict protective immunity against Erysipelas. This study aimed to update the knowledge on specific *E. rhusiopathiae* humoral immunity conferred by PARVORUVAX in naïve pigs.
Materials and Methods: Seven SPF pigs aged 10-15 weeks were vaccinated twice four weeks apart (D0, D28) with PARVORUVAX, according to the recommendations of the manufacturer. A second group of two non-vaccinated SPF piglets was used as non-vaccinated control group. Sera were collected on D0 then on a weekly basis from D28 to D49. The humoral immunity was assessed by antibody titration both using a commercial indirect ELISA kit (Ingezim® Mal Rojo, Ingenasa, Spain) according to the manufacturer specifications and a rSpaA415 ELISA performed at the Roslin Institute, Scotland, UK.
Results: Sera of control pigs remained negative using the two analytical techniques. On D28, as soon as 4 weeks after initial vaccination, a clear and significant ($p<0.01$) humoral anti-SpaA response was observed in 100% (7/7) of the vaccinated pigs. In addition, after booster vaccination a significant increase in the specific SpaA antibody titres was seen ($p<0.01$).
With the commercial ELISA kit, the detection of the humoral response appeared to be slower: 3 negative, 3 doubtful and 1/7 positive sera were found on D28. From D35, i.e. from one week after the second injection, 7/7 vaccinates were found positives.
Using OD values, the correlation between the two titration techniques was moderate (Pearson's correlation coefficient = 0.73).
Conclusion: Under the conditions of the study, PARVORUVAX induced an early and clear humoral immunity against SpaA which is known to be likely linked with protection. The discrepancy between the kinetic of antibody induction evaluated by the two techniques maybe due to the fact that the commercial kit is not tightly related to the detection and titration of anti-SpaA antibodies but on other *E. rhusiopathiae* antigens.
®PARVORUVAX is a registered trademark of Merial.

Disclosure of Interest: S. Crussard Conflict with: Merial S.A.S., P. Gerber: None Declared, O. Merdy Conflict with: Merial S.A.S., F. Joisel Conflict with: Merial S.A.S., T. Opriessnig: None Declared, C. Charreyre Conflict with: Merial S.A.S.

Keywords: Erysipelothrix rhusiopathiae, SpaA, Vaccination

Poster Abstracts

Bacteriology and Bacterial Diseases
OTHERS

PO-PF3-038

Characterization of Pasteurella multocida isolates obtained from swine in central area of Argentina

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Introduction: *Pasteurella multocida* (Pm) is an important organism in the etiological complex of swine pneumonia. Pm is a ubiquitous agent often isolated from nasal cavities of pigs with or without clinical signs of atrophic rhinitis, from tonsils and/or from lungs of pigs with or without clinical signs of pneumonia. In our country there is a lack of information about the characteristics of Pm in swine in spite of previous studies have been carried out (Leotta et al., 2006; Moredo 2008). Thus, the objective of this study was to characterize Pm isolates obtained from nasal cavities, tonsils and lungs of pigs with or without respiratory clinical signs.
Materials and Methods: DNA from all the 67 Pm strains (n=8 from nasal cavity, n=5 from tonsils and n=54 from lungs) stored in our Laboratory, was extracted by boiling. In order to characterize the isolates 3 PCRs were carried out. First, the reaction described by Register et al., (2006) to confirm the species (KMT gene), second PCR described by Townsend et al. (2001) to identify the capsular serotype (A or D) and finally the reaction described by Nagai et al. (1994) to identify toxigenic Pm strains.
Results: All the 67 isolates were positives to a specie-specific PCR (KMT gene). Serotype A was found in 88% (59/67) of the isolates: 8.5% (5/59) from nasal cavity, 6.7% (4/59) from tonsils and 84.7% (50/59) from lungs. Serotype D was found in 7.5% (5/67) of the isolates: 40% (2/5) from nasal cavity and 60% (3/5) from lungs. Three isolates could not be typed. None of the isolates was positive to *toxA* gene being some of these isolates from pigs with atrophic rhinitis.
Conclusion: Most of Pm isolates were identified as capsular serotype A. This is an expected result since most of them were isolated from lungs and serotype A is often collected from pneumonic process (Rutter, 1983; Borowsky et al., 2002). Both type A and D strains were present in the same proportion in the nasal cavities from pigs with or without clinical signs of atrophic rhinitis (data not shown). It seems there is not a predominance of one type over the other as previously reported (lariviere et al., 1992). None of the analyzed Pm strains was toxigenic in spite of had been isolated from pigs with clinical signs. While the frequency of Pm toxigenic could be low (Pijoan et al., 1983; Borowski et al., 2002) or high (Sarangi et al., 2014), in our country there is no report of toxigenic strains. More studies should be conducted in order to determine the frequency of toxigenic Pm strains in our region.

Disclosure of Interest: None Declared

Keywords: capsular serotype, Pasteurella multocida, toxigenic

Bacteriology and Bacterial Diseases
OTHERS

PO-PF3-151

ANTIBODIES AGAINST Leptospira spp. IN SOWS AND PIGLETS IN COMPLETE CYCLE OF PIG PRODUCTION, LOCATED IN ARAPONGAS-PR.

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Introduction: Leptospirosis in swine is worldwide known as the major cause of economic losses due to reproductive problems. The serovars Canicola, Pomona and Icterohaemorrhagiae are often identified as cause of illness in this species. In the complete system of pig production, the high density of animals per square meter may favor the transmission of the etiologic agent and increase the risk of infection. Thus, the objective of the study was to determine the frequency of reactive animals to *Leptospira* spp., regarding sows and piglets, in a complete system of pig production, located in Arapongas, Paraná.
Materials and Methods: A total of 58 serum samples were used, being 12 from sows and 46 from piglets, being four piglets from each sow. The sows were vaccinated and the blood sampling was collected four weeks before parturition, and from the piglets was collected at seven days of age. The diagnosis was made by the Microscopic Agglutination Test (MAT), against a battery of 24 serovars of *Leptospira* spp. We considered positive the animals with titer ≥100 in the MAT. To observe the prevalence, we considered positive the animals reactive to one or more serovars. Moreover, for the determination of the most probable serovars, we considered only the serovar with the highest titer, and the animals with titer against two or more serovars were disregarded.
Results: Among the analyzed sows, 83.33% (10/12) were seropositive to at least one serovar of *Leptospira* spp., and the titers ranged from 100 to 800 in MAT. The serovar most probable of causing infection was the Icterohaemorrhagiae with 44.44% of seropositive animals, followed by Pomona (33.33%) and Wolffi (22.22%). Regarding the analyzed piglets, no animal was seropositive in the Microscopic Agglutination Test.
Conclusion: Leptospirosis in swine is still a common disease. The presence of animals reactive to serovar Icterohaemorrhagiae in this study suggests a previous contact with rodents, since they are the major reservoir for this serovar. This study reinforces the importance of implementing health-management programs in pig farms, as this serovar is related to diseases in humans, thus becoming a problem of public health. A longitudinal study is required to show the main serovars in each stage of the pig production cycle, to obtain better control of the disease within farms, improving biosecurity.

Disclosure of Interest: None Declared

Keywords: biosecurity, leptospirosis, swine

Bacteriology and Bacterial Diseases
OTHERS

PO-PF3-006

Erysipelothrix spp. serotypes commonly identified in pigs in Great Britain during 1987-2015

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Introduction: *Erysipelothrix spp.* is the causative pathogen of swine erysipelas. Pigs infected with *Erysipelothrix* spp. can show a range of clinical signs including sudden death, fever, skin discolorations and lameness. *Erysipelothrix* spp. is also a zoonotic pathogen and is considered an occupational disease putting farmers, veterinarians and butchers at highest risk. There are at least 28 different *Erysipelothrix* serotypes, 1a, 1b, and 2 being the most common and most virulent in pigs. All of these serotypes belong to *Erysipelothrix rhusiopathiae* Available licenced vaccines in Great Britain protect pigs against *E. rhusiopathiae* are all inactivated and based on serotype 2. In recent years there have been anecdotal reports of increased diagnoses of erysipelas and there are concerns that current vaccines do not fully cross-protect against potential emerging *Erysipelothrix* spp. strains. The objective of this study was to characterize recent GB *Erysipelothrix* spp. isolates by serotyping to identify potential trends.
Materials and Methods: A total of 153 *Erysipelothrix spp.* isolates cultured from pig tissues in submissions to APHA/SAC CVS (for diagnostic investigations incuding for suspected erysipelas) during 1987 to 2015 from various British pig farms were processed for serotyping according to standard protocols. After inactivation, the serotype of each isolate was determined using agar gel immunodiffusion tests.
Results: Of the 153 isolates, 56.2% (86/153) were serotype 2, 17.6% (27/153) were serotype 1a, 15% (23/153) were serotype 1b and 2.6% (4/153) were serotype 11. The remaining 7.2% were serotypes 5 (3/153), 9 (1/153), 10 (3/153) and 15 (3/153). All of these serotypes belong to the *E. rhusiopathiae* genotype. Three were untypeable.
Conclusion: In this study the main *E. rhusiopathiae* serotype identified in British pig tissues was serotype 2 which is also the serotype present in current UK vaccines. Further studies need to investigate differences between the vaccine strains and current field isolates to determine if antigenic changes have evolved that may interfere with vaccine protection. The serotypes in outbreaks in vaccinated pigs also need to be compared with serotypes from outbreaks in non-vaccinated pigs. In addition, compliance with vaccine usage guidelines, in particular vaccine storage, timing and administration needs to be revisited on affected farms.

Disclosure of Interest: None Declared

Keywords: Erysipelothrix rhusiopathiae, Great Britain, Serotypes

Bacteriology and Bacterial Diseases
OTHERS

PO-PF3-049

Study of seronegative subpopulations against Swine Parvovirus (PPV), Swine Erysipelas (SE), and Swine Leptospira (SL) in 21 swine Brazilian herds.

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Introduction: Protection against PPV, SL, and SE comes mainly from the humoral immunity induced by the consecutive vaccinations. Therefore when a serologic analysis by means of a serumprofile is performed in a vaccinated breeders herd, it's expected that most of the sows are seropositive.
The objective of this study is to determine the existence of seronegative subpopulations against these 3 reproductive diseases in 21 Brazilian farms which are vaccinating routinely with a commercial trivalent vaccine (Parvovirus, Erysipela and Leptospira).
Materials and Methods: 751 blood samples were collected from 21 farms. 30-40 blood samples were collected per farm (depending on farm size). Farms were located in the Brazilian states of Minas Gerais, Paraná, Santa Catarina and Rio Grande do Sul. All the sampled animals (sows and gilts) had been vaccinated previously with a trivalent vaccine which in its composition included; *Erysipelothrix rhusiopathiae* , Porcine Parvovirus and Leptospira (*L.canicola, L.grippothiposa, L. Hardjo, L. Bratislava, y L.Pomona*).
Samples from every farm were stratified by five parity groups: gilts, parity 1-2, parity 3-4, parity 5-6 and more than 7. Breeder's blood samples were collected around 60-80 days of gestation.
Serology for PPV (Hemaglutination inhibition test), Leptospira (Plate microaglutination against 8 serovars) and SE (CIVTEST ELISA suis ERY) was performed following standard protocols.
Results: SE:34% of tested samples were seronegatives. Particularly, 48% of gilts were seronegatives. No significant differences were observed among farms.
PPV:93% of global seropositivity was observed. Besides, 19% of total gilts tested were seronegative. The high percentage of seropositive sows observed can be explained due to the wild virus circulation in most of the Brazilian farms.
Leptospira sp.:61 % of tested animals were seropositive at least for one Leptospira serovar. Percentages of seropositivity per serovar were 39% *L. Icterohaemorrhagiae;* 24% *L. Grippotyphosa;* 18 % *L. Pomona;* percentages of seropositivity for the other serovars were less than 15%.
Conclusion: In this serologic study, the high proportion of seronegative breeders subpopulations is very relevant, and so there are breeders partially unprotected against SE disease and PPV disease, in spite of these animals have been vaccinated with a commercial vaccine. This situation is more pronounced in gilts. Besides, very low serologic response against Leptospira serovars present in the vaccine is observed. This study confirms the high prevalence of seronegative breeders for SE and PPV in Brazilian farms, as previous studies described.

Disclosure of Interest: None Declared

Keywords: Erysipelothrix rhusiopathiae, Leptospira sp, Porcine parvovirus

Poster Abstracts

Bacteriology and Bacterial Diseases
OTHERS

PO-PF3-019

Evaluation of four ELISA assays to diagnose Mycobacterium tuberculosis complex infection in pigs

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Introduction: In countries in which bovine tuberculosis (bTB) is still prevalent or is re-emerging the contact among different animal species in extensive systems may contribute to the circulation of *Mycobacterium bovis* and other members of the *Mycobacterium tuberculosis* complex (MTC) and the spread of this disease. Thus, free-range pigs may be infected by MTC, developing subclinical infections, which are not detected until meat inspection procedures at slaughterhouse. Serodiagnosis has been recently proposed as a reliable screening tool for detecting infected herds. In this study four ELISA assays using different *M. bovis* peptides/proteins (MPB70+MPB83, INGENASA; treated bovine purified protein derivative, t-bPPD; bPPD1; and bPPD2 VACUNEK) as coating antigens were evaluated to diagnose MTC infection in pigs.
Materials and Methods: Submandibular lymph nodes (SLN) and blood samples from 129 free-range pigs raised on Southern Spain farms with a history of condemnation due to tuberculosis-like lesions were sampled at slaughterhouse. SLN were tested by gross examination, histopathology, bacteriological culture and qPCR. Ninety-seven out of these animals were classified as bTB positive cases (compatible lesions and MTC detection by means of culture and qPCR) or bTB negative cases (absence of compatible lesions and negative MTC detection) and used as reference method. When necessary different cut-off values were evaluated.
Results: All assays had a very good concordance between them (k ≥ 0.82). The MPB70+MPB83 based ELISA had the best sensitivity (Se) (78%, CI95 67.4%>88.5%) and a good concordance with the reference method (k=0.69). The t-bPPD and the bPPD1 in-house assays presented a slightly reduced Se (71.2%, CI95 59.6%>82.7%; and 66.1%, CI95 54%>78.2%; respectively) and a moderate concordance with the reference method (k=0.57 and 0.52, respectively). When the bPPD2 based ELISA was evaluated, similar Se to the previous ones was obtained using a cut-off of 0.35 (Se: 66.1%, CI95 54%>78.2%; k=0.52).
Conclusion: These results suggest that despite the fact that MPB70+MPB83 ELISA presented the best results all four evaluated ELISA assays could be used as a screening tool to conduct TB surveillance in pigs at a population level. In addition, a cut-off of 0.35 is recommended for bPPD2 ELISA in order to obtain better diagnostic values.

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Disclosure of Interest: None Declared

Keywords: diagnosis, ELISA, Mycobacterium tuberculosis complex

Bacteriology and Bacterial Diseases
OTHERS

PO-PF3-030

Pathogenicity investigation of a pig-sourced Pasteurella multocida serotype F isolate in different susceptible animals

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Introduction: *Pasteurella multocida* is an important contributory agent of porcine respiratory diseases. There are five *P. multocida* serogroups (A, B, D, E and F) according to the differences in their capsule antigen. Of which, serogroup F isolates are predominately prevalent in avian hosts, but rarely seen in pigs. Moreover, little is known about the virulence of this serotype, especially those sourced from pigs. We have recovered several field isolates of *P. multocida* serotype F from pigs in China. To understand the pathogenicity of these strains, one of the serotype F isolates, designated HN07, was used to infect animals susceptible to *P. multocida.*
Materials and Methods: In mice experiment, different groups of mice were challenged intraperitoneally with bacterial suspensions of HN07 and a toxigenic *P. multocida* HN06 at ~10^3 CFU, ~10^2 CFU and ~10 CFU, respectively; in chicken experiment, different groups of chickens were challenged intratracheally with HN07 at ~10^9 CFU and ~10^4 CFU, respectively. As a comparison, the control chickens were challenged with a fowl cholera isolate GX at the same doses; in rabbit experiment, the experimental rabbits were challenged intranasally HN07 at ~10^5 CFU; in pig experiment, the experimental pigs were challenged intratracheally HN07 at ~10^{10} CFU. The negative control animals in the study were challenged with sterile 0.9% normal saline. Clinical signs were monitored daily after challenge. Histological damages were examined via Hematoxylin-Eosin staining. Bacteriological examination was performed using PCR. (*This study was approved by the Ethical Committee for Animal Experiments at Huazhong Agricultural University, Wuhan, China.*)
Results: Strain HN07 show higher pathogenicity to mice compared to the toxigenic strain. However, it did not induce severe clinical signs in experimental chickens even at an infective dose of ~10^9 CFU. Interestingly, this strain caused severe clinical signs that ultimately led to death in the rabbits challenged with ~10^5 CFU; the main pathological changes noted were characteristic of fibrinopurulent pneumonia and hemorrhagic pneumonia. As expected, the strain led to the clinical signs and the pathological lesions in experimental pigs that are similar to the pasteurellosis disease.
Conclusion: This work reveals the high pathogenicity of the pig-sourced *P. multocida* serotype F to mice, rabbits, and pigs, but a low virulence to chickens. A small number of studies reporting the isolation of *P. multocida* serotype F of porcine source exist, but the present study is the first to report on the pathogenicity of *P. multocida* of serotype F of pig origin. Our findings advance current knowledge of the pathogenesis profiles of *P. multocida* type F strains.

Disclosure of Interest: None Declared

Keywords: Pasteurella multocida; serotype F; pathogenicity

Bacteriology and Bacterial Diseases
OTHERS

PO-PF3-012

Virulence gene profiling of Pasteurella multocida isolates from pigs and wild boars

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Introduction: *Pasteurella multocida,* a widespread Gram-negative bacterium, is a common inhabitant of the upper respiratory tract of several avian and mammalian species causing various, frequently host specific diseases. In pigs, *P. multocida* is associated with atrophic rhinitis and pneumonia causing remarkable economic losses for swine production worldwide. *P. multocida* produces a number of virulence factors, including toxin production, various adhesins and siderophores that may play a role in colonization and invasion of the host. The aim of our study was to obtain data about the occurrence of selected virulence genes in *P. multocida* strains isolated from pigs and wild boars.
Materials and Methods: A total of 38 isolates of *P. multocida* recovered from diseased swine and wild boars were examined. Following species-specific identification, the strains were screened by PCR for the presence of capsule biosynthesis genes and 9 virulence associated genes. Combinations of oligonucleotide primers were used for amplification of *kmt1* (species identification) and *toxA* (*P. multocida* toxin) sequences in the same reaction. The capsular type was identified using a multiplex PCR method, as described in the literature. To determine virulence associated genes, multiple adhesins were tested: type I and type IV fimbrial subunits (*fimA, ptfA*), autotransporter adhesins (*hsf1, hsf2*), tight adherence protein D (*tadD*) and filamentous haemagglutinin (*pfhA*). Presence of iron acquisition proteins (*tbpA, hgbB*) was also analysed.
Results: The most prevalent capsular type detected in *P. multocida* strains from pigs was type A (72.4%). Capsular type D (20.7%) and F (6.9%) were also identified. All isolates from wild boars gave positive results by the PCR assays for capsular type D. *ToxA* was obtained from 41.4% of pigs, and 88.9% of wild boars. Of the adhesion-encoding genes, *fimA*, *hsf2* and *ptfA* were each found to occur in 100% of the strains. *Hsf1* was detected from 69.0% (pigs) and 100% (wild boars) of the isolates. In contrast, *pfhA* and *tadD* genes were found in lower prevalence (27.6% and 34.5%) from pig isolates, and were not detected in stains from wild boars. *HgbB* was found in more than 75% of the isolates, and none of the strains harboured the *tbpA* gene.
Conclusion: This study provided useful epidemiological information on the prevalence of various virulence factors of swine and wild boars in Hungary. In addition to the expansion of scientific knowledge, results of this research might help to elucidate the mechanisms of pathogenesis and improving diagnostic methods.

Disclosure of Interest: None Declared

Keywords: Pasteurella multocida

Bacteriology and Bacterial Diseases
OTHERS

PO-PF3-022

Antibiotic susceptibility and PCR-RFLP patterns of Bordetella bronchiseptica strains isolated from pigs and other hosts in Hungary

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Introduction: *Bordetella bronchiseptica* (Bb) is involved in the aetiology of atrophic rhinitis of swine and causes remarkable economic losses for pig production. Antibiotic treatment is widely used for the treatment of bacterial infections, nevertheless, little is known about the antibiotic resistance of the *B. bronchiseptica* strains circulating in Hungary. Our aim was to fill this gap by a retrospective analysis of strains originated from pigs. Furthermore, we determined the PCR-RFLP patterns of some strains and compared to those of strains from other host species.
Materials and Methods: Antibiotic susceptibility of 15 Hungarian strains was determined by Kirby-Bauer disk diffusion method. Plasmid isolation was performed with QIAprep Spin Miniprep Kit. PCR-RFLP: 43 Bb isolates from pigs, 38 from dogs, 4 from cats were analysed on *fim*A by *Hinc*II and *Sal*I, on *fla*A by *Bgl*I, *Hinc*II and *Msp*I, and on *cya*A by *Nar*I and *Sal*I.
Results: All strains were susceptible to colistin and amphenicols, but a high levels of resistance were detected to a number of other antibiotics (penicillin, vancomycin, lincomycin and ceftiofur). The resistance to ampicillin, neomycin, tilmicosin and flumequine varied widely among the strains. All but five of the strains were susceptible to sulphonamides while only one isolate was resistant against tetracycline and nalidixic acid, respectively. Four out of 15 strains presented plasmids. The PCR-RFLP of *fim*A resulted uniform bands in all strains examined. By PCR-RFLP of *fla*A, the isolates from pigs in Hungary were uniform and differed from strains originated from the other hosts, while the isolates from pigs from other countries represented three types (A, B and C). Most strains of porcine origin (93%) belonged to the type B. The PCR RFLP of *cya*A of strains from pigs proved to be uniform (pattern A), only one foreign porcine isolate belonged to type D.
Conclusion: The practice of antibiotic therapy in livestock farms might affects the rate of resistance against antibiotics. However, our study revealed no correlation between the antibiotic resistance patterns and the origin of the strains. Signs of general antibiotic resistance spreading were not detected. The present results point to a possible connection between Bb *fla*A PCR-RFLP profiles and hosts. In a certain geographical region, signs of host adaptation were recognizable.

Disclosure of Interest: None Declared

Keywords: Bordetella bronchiseptica

Poster Abstracts

Bacteriology and Bacterial Diseases
OTHERS

PO-PF3-031

Prevalence of antibodies to selected Leptospira serovars in swine from Poland

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Introduction: Leptospirosis is a worldwide zoonotic disease caused by pathogenic serovars of *Leptospira* sp. The disease in swine is mainly transmitted by contact with infected urine, blood, tissues, organs, contaminated water and feed. Leptospirosis can be spread by rodents or by direct contact of infected pigs. Clinical symptoms such as abortion, stillbirths and sometimes infertility are the most often observed consequences of *Leptospira* sp. infection in swine. They cause serious economic losses and may pose a threat to farm staff taking care of the animals. The aim of the study was to examine the seroprevalence of *Leptospira* sp. infections in Polish swine population.
Materials and Methods: A total of 22883 swine serum samples (up to 400 serum samples for one province per year) were collected from 2011 to 2015 in the monitoring program from all 16 provinces of Poland. All the serum samples were tested by microscopic agglutination test (MAT) with the panel of 6 serovars representative of 6 serogroups, most often found in swine population in Poland: Icterohaemorrhagiae (RGA), Grippotyphosa (Moskva V), Sejroe (M84), Tarassovi (Perepelicyn), Pomona (Pomona), Canicola (Hond Utrecht IV). The minimum sera dilution was 1:100.
Results: During the last 5 years the seroprevalence of *Leptospira* in swine was as follows: 2.68%, 1.47%, 2.02%, 1.42% and 1.32%, respectively. Yearly, the highest percentages of seropositive swine serum samples, were found in the north and south part of the country, while a low seroprevalence was observed in the central and western regions of Poland. The most common serovars between 2011-2015 were: Pomona (1.01%, 0.39%, 0.62%, 1.02% and 1.13%, respectively) and Sejroe (1.12%, 0.8%, 1.05%, 0.36% and 0.18%, respectively).
Conclusion: Similar studies conducted in 2009-2010 showed a slightly lower seroprevalence, of around 1%, with regard to the tested *Leptospira* serovars. Pomona and Sejroe have been constantly, the most common serovars isolated from pigs in Poland. Our observations from recent two years confirm that serological reactions with serovar Sejroe in pigs in Poland have a decreasing tendency. The occurrence of other *Leptospira* serovars is marginal. Although west and north-west part of the country represent a high pig population density and an intensive pig production system, our investigations from the last years indicate a low seroprevalence to 6 tested serovars. Described above regions with the highest *Leptospira* seroprevalence represent more extensive pig production systems.

Disclosure of Interest: None Declared

Keywords: Leptospira sp., MAT, prevalence

Bacteriology and Bacterial Diseases
OTHERS

PO-PF3-032

INCREASED PREVALENCE OF LEPTOSPIROSIS SINCE THE GROUPING OF SOWS: MYTH OR REALITY?

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Introduction: It is still controversial among French veterinarians whether leptospirosis has a role in some cases of infertility in pork production, The veterinarians of SOCAVET describe three experiments implemented to prove the real involvment of the Leptospires in some reproductive failures , and the increase of cases after the grouping of the sows in 2013 . The first experiment was a vaccination trial performed in 1999, already reported in IPVS 2000, where fertility and abortions had been reduced by vaccination. The second repport is a chronic infection case certified by PCR with re-infection at the end of each antimicrobial treatement, and the third element is the analysis of the evolution of reproductive performances after the antimicrobial treatment, in 15 farms detected as serologically positive by the micro-agglutination test : the fertility, abortion and farrowing rates are all improved by treatment, but productivity parameters are not modified.
Materials and Methods: The veterinarian group SOCAVET conducted serological screening of leptospirosis in 49 farrow-to-finish farms with fertility problems, abortions or stillbirths in 2014 and studied the evolution of zootechnical parameters in the period 4 months prior to and 4 months after treatment on 14 farms: fertility rates , number of abortions, farrowing rate and productivity .
In 2015 N. PEREZ diagnosed a case of leptospirosis by PCR in mummified piglets ,despite negative serology on a farm of 80 sows
Results: The leptospirosis case study interrogates us on the possible insensitivity of the MAT method in certain infected farm, and shows in the field the ability of leptospires to remain dormant in internal organs during treatment with antibiotics then clinically resurface fairly soon after the end of treatment.
The analysis of the evolution of reproductive performances after the antimicrobial treatment, in 15 farms detected as serologically positive by the micro-agglutination test shows that fertility rate, abortion and farrowing rates are all improved by treatment, but productivity parameters are not modified.
Conclusion: The role of leptospires in swine infertility in France should no longer be the subject of controversy. However diagnostic tools need to be improved to ensure the quality of disease diagnosis in the field due to the uncertainty between serology and PCR, and between contact and disease.

Disclosure of Interest: None Declared

Keywords: france, leptospirosis, sows

Bacteriology and Bacterial Diseases
OTHERS

PO-PT2-001

FUSOBACTERIUM GASTROSUIS SP. NOV.: A MAJOR COMPONENT OF THE GASTRIC MICROBIOTA OF PIGS WITH GASTRIC ULCERS

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Introduction: *Helicobacter suis* may play a role in gastric ulcer disease, possibly by affecting gastric acid secretion and by modifying the composition of the gastric microbiota. In a recent metagenomics study, an unidentified *Fusobacterium* sp. was present in higher numbers in the stomach of *H. suis*-infected than non-infected pigs. Here, we describe the isolation and characterization of this new species.
Materials and Methods: Sixty stomachs from 6-8 months old pigs and adult sows were collected in slaughterhouses. Swabs were taken from each stomach region and streaked on Columbia agar plates®, supplemented with 5% sheep blood, neomycin, vancomycin and erythromycin. After anaerobic incubation during 3 days, 9 isolates of a putative new *Fusobacterium sp.* were obtained. All isolates originated from pigs with ulcers. Seven isolates were obtained from the pars oesophagea, one from the cardia and one from the antrum. All isolates were phenotypically and genotypically characterized.
Based on the *gyrase B* gene, a species-specific qPCR was developed to quantify the numbers of this *Fusobacterium* species in different regions of the stomach of pigs with and without ulcers.
Results: All isolates formed circular, white colonies of approximately 0.4 cm in diameter that were surrounded by a narrow zone of complete hemolysis. The isolates were obligately anaerobic, although they tolerated 2 hours exposure to air. Gram-staining revealed 1.5-2 µm long and 0.3-0.5 µm wide Gram-negative rods with rounded ends. The *16S rRNA* gene sequence showed 96% similarity with *F. mortiferum* and 95% with *F. necrogenes*, its closest phylogenetic neighbours. For the *gyrase B* gene sequence this was 74% and 73%, respectively. The novel *Fusobacterium* sp. was nonfermentative, indole positive and did not hydrolyse esculin, whereas *F. mortiferum* and *F. necrogenes* are weakly fermentative, indole negative and do hydrolyse esculin. The major fatty acids were C16 : 0 and C18 : 1ω9c.
As determined by qPCR, colonization levels were highest in the pars oesophageal region and a positive correlation was found between gastric ulceration and colonization levels.
Conclusion: The novel species, for which we propose the name *Fusobacterium gastrosuis*, can be clearly differentiated from its nearest phylogenetic neighbours. Whole-genome sequencing and quantification of colonization levels in a larger number of animals are currently being performed. This, as well as experimental infection studies, should allow to obtain better insights in the potential role of this microorganism in the development of gastric pathologies in pigs, since most known *Fusobacterium* species are indeed considered to be pathogens.

Disclosure of Interest: None Declared

Keywords: Gastric ulcers, microbiota, Fusobacterium

Bacteriology and Bacterial Diseases
OTHERS

PO-PF3-027

Ultraviolet (UV-C) inactivation of Enterococcus faecium as surrogate bacterial pathogen model in liquid porcine plasma

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Introduction: Spray dried plasma (SDP) is a functional protein source used in pig diets due to its beneficial effects on post-weaning performance and survival. Although the manufacturing process of SDP involves several safety features including collection from healthy animals, pooling, and spray drying at high temperature, as technologies develop, additional safety features should be investigated.Ultraviolet at 254 nm wavelength (UV-C) is a non-thermal process that disrupts cellular transcription and replication leading to death of microorganisms. *Enterococcus faecium* (*E. faecium*) NRRL B-2354 has been used as a model organism in thermal validation studies and is considered a suitable surrogate for foodborne pathogens to validate thermal processes used for dairy products, almonds, liquid foods and meat. Therefore, the objective of this study was to evaluate the effectiveness of UV-C irradiation using a proprietary system (SurePure SP1) on survival of *E. faecium* in liquid porcine plasma.
Materials and Methods: Spray dried porcine plasma (2.5 Kg) was irradiated at 10 KGy to eliminate any potential bacteria. The irradiated spray-dried porcine plasma was diluted 1:11 (2.5 Kg spray-dried plasma + 25.0 Kg of water) to obtain 27.5 Kg of liquid plasma at 9.11% solids. Then, 24 L of the diluted porcine plasma was divided in three different sub-batches of 8 L each. At time zero, 15 mL samples were obtained and served as negative control before bacteria inoculation. A positive control sample was collected 5 min after the fresh plasma was inoculated with *E. faecium*. Each sub-batch was consecutively irradiated at 1500, 3000, 6000 and 9000 J/L. Plasma was recirculated under turbulent flow at 4000 L/h in a closed system of the SP1 device. During the UV treatment, sequential samples were taken at each dose of UV irradiation. After UV irradiation, samples were log diluted in peptone water and 0.1 mL inoculated in BHIA for 24 h at 37ºC and analyzed for colony forming units (CFU) counts.
Results: Liquid initial plasma was inoculated with 6.22 ± 0.14 Log_{10} cfu *E. faecium* /mL (mean ± SD). Results indicated a reduction of 1.01, 3.70, 5.61 and >6.22 log_{10} at 1500, 3000, 6000 and 9000J/L, respectively, for *E. faecium*.
Conclusion: Overall results indicated a reduction of *E. faecium* when submitted to UV irradiation. Four log reduction was obtained by an UV dose close to 3000 J/L. Taking into account *E. faecium* as a general surrogate marker for bacteria, these results indicate the usefulness of the UV treatment to inactivate food borne bacteria in liquid plasma as an intermediate additional safety feature for the manufacturing process of spray-dried plasma.

Disclosure of Interest: E. Blázquez Conflict with: APC EUROPE, Conflict with: APC-EUROPE, J. Ródenas Conflict with: APC EUROPE, C. Rodríguez Conflict with: APC EUROPE, J. Segalés: None Declared, J. Pujols: None Declared, J. Polo Conflict with: APC-EUROPE

Keywords: Enterococcus faecium , spray dried plasma, Ultraviolet UV-C

Poster Abstracts

Bacteriology and Bacterial Diseases
OTHERS

PO-PF3-013

Identification of a Mycobacterium avium high risk pig farm with antibody detection by ELISA

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Introduction: For export of breeding sows and boars, a status of freedom from Mycobacterium avium (M. avium) of the exported animals is demanded in some countries. Usually, testing is done by tuberculin test of each animal before transport. Aim of this study was to determine if the identification of M. avium high risk farms is possible by detection of M. avium specific antibodies.
Materials and Methods: Breeding pigs from an Austrian pig farm were found positive by tuberculin test. A granulomatous lymphadenitis of the mesenterial lymph nodes caused by M. avium subspecies hominissuis was detected after slaughtering of the suspected pigs by PCR and bacteriological culture. Serum samples of 128 pigs from this farm aged about seven month that were confirmed to be M. avium positive were collected. As a control group, 87 serum samples of pigs of the same age from a M. avium unsuspected Austrian farm were taken. All 215 serum samples were analyzed by the PrioCHECK® M. avium Ab porcine ELISA for presence of M. avium specific antibodies.
Results: Out of the 128 samples of the pigs from the M. avium positive farm, 39 (30.5%) were found M. avium antibody positive, 42 (32.8%) were questionable and 28 (45.0%) were negative. Out of the 87 samples from the unsuspected farm, two (2.3%) were antibody positive, four (4.6%) were questionable and 81 (93.1%) negative. Therefore, the specificity of the ELISA, determined with the data of the pigs from the unsuspected farm, was calculated to be 0.93 (95% confidence interval 87.8%; 98.4%).
Conclusion: With detection of M. avium specific antibodies by ELISA, the identification of high risk farms at farm level is possible. The production of M. avium specific antibodies is only induced by severe organ manifestation in the respective pig. Therefore, the individual diagnosis based on ELISA testing is not recommendable. Furthermore, an improvement of sensitivity and specificity of the ELISA is necessary, especially to be able to declare non-suspect farms.

Disclosure of Interest: None Declared

Keywords: antibodies, Mycobacteriosis, tuberculin test

Bacteriology and Bacterial Diseases
OTHERS

PO-PF3-026

Disease patterns, hygiene and climatic factors in two pig herds with registrations for respiratory lesions at slaughter

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Introduction: Increasing herd sizes may lead to more complex pathways for transmission of respiratory pathogens including secondary invaders. The aim of this study was to investigate the presence and impact of *Actinobacillus pleuropneumoniae* (App) and *Mycoplasma hyopneumoniae* (Mhyo) with that of the secondary invaders *Pasteurella multocida* (Pmt) and *Streptococcus suis* (Sss) in pig herds with high incidences of respiratory lesions at slaughter. In addition, hygiene and climatic factors were registered.
Materials and Methods: Two batches of growers (25-120 kg) were followed in each of two farrow-to-finish herds with high incidences of respiratory lesions at slaughter. Blood samples were collected from 12 pigs in each batch at week 0, 4 and 8, and analysed for antibodies to Mhyo, App2 and 3, Pmt and Sss, as well as for haematological parameters. Data regarding pen hygiene and climatic factors were recorded and Temperature Humidity Indexes (THI) were established.
Results: Hb concentrations ranged from 100-125 g /L. White Blood Cell (WBC) counts ranged from 23.5- 27.1 x 10^9 per L week 0, which ceased to a range of 19.7-23.1 after 8 weeks.
Herd A: In batch 1, pigs were seropositive to Pmt in both batches and to App2 at week 0. In batch 1 they seroconverted to App2 after 4 weeks. In both batches, the pigs had started to seroconvert to Mhyo by week 8. No reactors to Sss. In batch 2, the indoor THI was 19, 24 and 21 at 0, 4 and 8 weeks, respectively. The pen hygiene decreased with increasing THI.
Herd B: In batch 1, pigs had seroconverted to App2 (all) and to Pmt (7/12) at week 8. In batch 2, 7/12 pigs were seropositive to Pmt and none to App2 at week 8. No reactors to Mhyo or Ssuis. In batch 2, the THI index was 18, 16, and 17 at 0, 4 and 8 weeks, respectively, and an initially poor pen hygiene improved with time.
Conclusion: The incidence of respiratory lesions at slaughter remained high in both herds despite a fair hygiene and a fair climate, although high THIs tended to reduce the hygiene. The initially high WBC counts indicated that pigs had been colonised prior to the transfer to the fattening units. Indeed, the total number of WBCs actually decreased during the fattening period, despite that antibodies levels to App, Pmt and Mhyo increased. Sss did not appear to play a role in these herds, and transmission of Mhyo took place later during rearing. The pathogen load varied somewhat between batches within herds. Despite high App titres in three out of four batches, Pmt appeared to be dominant in these two herds. As Pmt is a secondary invader, other infections not yet determined most likely also contributed to the lung lesions registered at slaughter.

Disclosure of Interest: None Declared

Keywords: None

Bacteriology and Bacterial Diseases
OTHERS

PO-PF3-042

Swine Helicobacteriosis: Injuries and immunohistochemistry in stomachs of piglets

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Introduction: Gastric ulcer is a major cause of sudden death in swine, being multifactorial, involving animals raised intensively and confined. The Helicobacter suis has been linked to this disease, thus becoming a major problem for pig farmers and industry. The aim of this study was to relate the pathological findings of subclinical gastric lesions that occur naturally in piglets, with the presence or absence of Helicobacter spp.
Materials and Methods: They used forty-eight piglets with an average weight of 33 kg and average of 78 days old, acquired in a commercial farm, which after slaughter have had their stomachs collected and evaluated. Sample aglandular, and glandular anatomical regions were collected for histopathologic and immunohistochemical evaluation.
Results: Macroscopically, 34 (70.83%) animals had lesions on aglandular region, while 14 (29.17%) nothing had. Microscopically, 44 animals (91.66%) showed parakeratosis. Of these, 22 had a discreet manner, 20 moderate and two severe. In the glandular region in 41 (85.4%) animals, there was a change in at least one of the three regions, and only seven animals (14.6%) showed no change in any of the three. The lesions were higher in antral regions and cardic, followed the fundus. In relation to immunohistochemistry, 21 animals were negative in all areas, 24 positive in at least one, and none were positive in all.
Conclusion: The pathological findings showed relationship with the bacteria, and its immunostaining not associated with gastric lesions in certain regions, demonstrates its saprophytic and opportunistic character.

Disclosure of Interest: None Declared

Keywords: Gastric ulcer, Helicobacteriosis, swine

Bacteriology and Bacterial Diseases
OTHERS

PO-PF3-014

Characteristics of Lactic Acid Bacteria Intended for Probiotic Use from Indigenous and Commercial Pig Feces in Thailand

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Introduction: Lactic acid bacteria (LAB) are currently applied as probiotics due to their ability to survive in gut environment and antagonistic effects toward enteric pathogens. Difference of species and strains of LAB directly relate to host type in each geographical area. However, there has still been inconsistency between genetic and phenotypic identifications. This study aimed to determine and validate identification methods for probiotic candidate selection between phenotypic; morphological and biochemical characteristics, protein profile analysis, genetic analyses; complete 16S rRNA gene sequencing and antimicrobial susceptibility using EFSA criteria.
Materials and Methods: 60 fecal samples were obtained from antibiotic-free healthy fattening indigenous pigs (N=30) and commercial pigs (N=30). LAB were isolated from the feces and screened for probiotics by acid and bile tolerance. The selected LAB were identified according to phenotypic and biochemical characterizations (Gram's staining, motility test, catalase production, ability to grow at 50ºC and 21 types of sugar assimilations), whole-cell protein profile by SDS-PAGE and complete 16S rRNA gene sequencing. The identified LAB were test suitability to use as probiotics following EFSA criteria using agar-disk diffusion test and minimum inhibitory concentrations (MICs).
Results: A total of 204 LAB were obtained from all pigs. 34 strains were selected as putative probiotics by screening criteria including acid and bile tolerance. According to SDS-PAGE and 16S rRNA gene sequencing, they were identified as *Enterococcus faecium* (N = 11), *Enterococcus hirae* (N =9), *Lactobacillus agilis* (N = 3), *Lactobacillus plantarum* (N = 4), *Pediococcus acidilactici* (N = 1), and *Pediococcus pentosaceus* (N = 6). After screening antimicrobial susceptibility by agar-disk diffusion test and confirming by MICs, only five isolates (1 *P. acidilactici*, 1 *P. pentosaceus* and 3 *L. plantarum*) from 34 selected LAB were suitable to use as probiotic.
Conclusion: This study recognizes that mannitol assimilation is the biochemical marker for differentiation of *E. faecium* and *E. hirae*, and growth ability at 50ºC to separate *P. pentosaceus* from *P. acidilactici*. By using SDS-PAGE, whole-cell protein patterns of LAB species-specific were demonstrated into different clades which agree with the phylogenetic tree of 16S rRNA gene sequencing; therefore it can be used as a consensual phenotypic screening tool for LAB species identification. The identification results reveal that *E. faecium* and *E. hirae* were the most prevalence putative probiotics in feces of indigenous pigs, whereas *P. pentosaceus* were the most common in feces of commercial pigs.

Disclosure of Interest: None Declared

Keywords: Characteristics, Pig feces, Probiotics

Poster Abstracts

Bacteriology and Bacterial Diseases
OTHERS

PO-PF3-011

Unusual pathogens – Case reports of 4 rare findings

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Introduction: Traditionally, bacteriological diagnostics are based upon colony-morphology and biotyping. During the later years, MALDI-TOF-identification has broadened the spectrum of bacteria that can be identified in laboratory submissions. Four case-stories on potentially important pathogens are presented.
Materials and Methods: Case 1: Massive problems with arthritis around weaning. 4 piglets were examined. Deeply cut teeth and swollen joints with fibrinous/purulent arthritis were observed. Four joints per piglet were examined bacteriologically.
Case 2: Many cases of arthritis during the first week of life. 3 piglets were examined (submitted with a time period of 14 days in between). Deep wounds on fore-knees, wound-infection after castration and fibrinous arthritis (2 of 3 piglets) were observed. Four joints per piglets were examined bacteriologically.
Case 3: Abortions one week before expected farrowing. 7 foetuses (from one abortion) were examined. Compact consistency of lungs and enlarged livers were observed. Lung-tissue from 3 foetuses was examined bacteriologically.
Case 4: Sudden deaths in growing pigs. One pig (20 kg) was examined. Valvular endocarditis was observed and heart-valves were examined bacteriologically.
Bacteriological examinations were carried out aerobically on meat-juice agars (In House (ox-heart, Peptone P (Oxoid), NaCL, Agar (Oxoid)).
Results: Case 1: In 8 joints (1-3 per piglet) moderate to massive growth of greyish, shiny colonies (1-3 mm in diameter) was observed in pure culture after 24 hours. MALDI-TOF identified the bacteria as *Actinobacillus equuli.*
Case 2: In 3 joints (0-2 per piglet) single colonies/ moderate growth of small alfa-haemolytic colonies were seen in a mixed flora after 24 hours. MALDI-TOF identified isolates from both laboratory submissions as *Aerococcus viridans*.
Case 3: In 2 of 3 lungs, massive growth of pinpoint, haemolytic colonies was observed in pure culture after 48 hours. MALDI-TOF identified the bacteria as *Trueperella abortisuis*.
Case 4: Pinpoint colonies were detected in a mixed flora after 24 hours. MALDI-TOF identified the bacteria as *Streptococcus gallolyticus* (previously: *Streptococcus bovis biotype 1*).
Conclusion: Traditionally, none of the presented bacteriae are considered important pathogens in pigs. Our findings indicate that they may be relevant to take into consideration. *Actinobacillus equuli* and *Aerococcus viridans* were previously isolated from arthritic joints in pigs and *Trueperella abortisuis* was previously isolated from aborted pig-fetuses and placenta. *Streptococcus gallolyticus* is seen in human endocarditis.

Disclosure of Interest: None Declared

Keywords: Actinobacillus equuli, Aerococcus viridans, Trueperella abortisuis

Bacteriology and Bacterial Diseases
S.SUIS

PO-PF3-127

Streptococcus suis autogenous vaccines in Dutch farms:
effect of sow vaccination on total mortality and antimicrobial usage in piglets after weaning

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Introduction: *Streptococcus suis* infections have a huge impact on animal welfare and economical performance in pig farms. Furthermore, they hinder the restrictive use of antimicrobials (AMB) because oral treatment is an important tool to control the disease.
Epidemiological studies have shown a high level of diversity in *S. suis* serotypes (st) between geographical regions and within farms. This has hampered the development and availability of effective commercial vaccines. Therefore, veterinarians also prescribe autogenous vaccines.
Only few experimental and even less field trials have been performed to study the efficacy of autogenous vaccines and the results are inconsistent. Extrapolation to an expected effect in a new field case is difficult because of the great variabililty in production methods, adjuvants, methods of isolation and selection of strains and vaccination protocols. In addition most studies concern st2 strains, while st9 strains are most prevalent on Dutch farms.
The objective of this multicentre retrospective cohort study is to investigate the effects of autogenous *S. suis* sow vaccines on total mortality (TM) and AMB use in weaned piglets, using a fixed protocol.
Materials and Methods: We included 8 farms (4158 sows) that were attended by vets from one clinic. These farms satisfied for all inclusion criteria. They started vaccination due to high mortality and high AMB use after weaning and were willing to participate. At least one st9 strain was present in the o/w adjuvanted vaccines which were produced at Biovac, France. Strains were isolated from piglets with typical signs. Sows were vaccinated 6-7 weeks pre-partum and boostered 4 weeks later. In the next cycle sows received only 1 booster 2-3 weeks pre-partum.
An avg. of 2.4 vaccine strains were selected out of 3-11 serotyped strains per farm. We investigated the results of TM and AMB treatment (amoxicillin and tmp/s p.o.) after weaning (4-10 weeks of age) during 6 months after the moment that piglets born from vaccinated sows reached the age of 10 weeks. These data were compared to data from 12 months earlier. For TM and AMB use we performed ANOVA analyses with month (nested in period), farm and period (pre and post) as categorical variables (SYSTAT for WINDOWS, Version 13.00.05).
Results: Mean TM after weaning was 4.05% pre- and 2.03% post-vaccination. This is a decrease of 50% ($p<0.001$). Oral AMB use in piglets was calculated as kg BW that could be treated/sow (present at the farms)/month. This resulted in 16.5 kg pre- and 6.90 kg post-vaccination which is a reduction of 58% ($p=0.016$).
Conclusion: In this study it was shown that autogenous *S. suis* sow vaccines play an important role in reduction of TM and AMB use in piglets after weaning.

Disclosure of Interest: B. Engelen Conflict with: Dopharma Research, B. Meyer Conflict with: Dopharma Research, V. Shankar: None Declared, V. Minten: None Declared

Keywords: autogenous vaccine, sow vaccination, Streptococcus suis

Bacteriology and Bacterial Diseases
S.SUIS

PO-PF3-105

Serotype, organic distribution and antimicrobial resistance of Streptococcus suis isolated from diseased pig in Taiwan

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Introduction: *Streptococcus suis* is a gram positive bacteria with capsule, which cause economic losses in swine industry. However investigations of serotype, organic distribution and antimicrobial resistance of *S. suis* in Taiwan was rare. This study was performed in order to establish diagnosis and treatment guidelines for clinic veterinarians by analysis current situation of *S. suis* in Taiwan.
Materials and Methods: In this study, 105 strains of *S. suis* were isolated from sick pigs which were sent to the Animal Disease Diagnostic Center of National Chiayi University in Taiwan from 2013 to 2015. The isolates were identified their serotypes by Polymerase Chain Reaction (PCR) and compared serotype with isolated site to statistic organic distribution. The minimum inhibitory concentration (MIC) test was performed according to Clinical and Laboratory Standards Institute operating rules. The following antimicrobial agents were selected for antimicrobial susceptibility testing: amoxicillin (AMO), cefazolin (CZ), ceftiofur (CT), doxycycline (DO), enrofloxacin (ENR), erythromycin (E), florfenicol (FFC), gentamicin (GN), lincomycin (L), lincospectin (LS), oxytetracycline (OTC), penicillin G (PG), sulfamethoxazole-trimethoprim (TS), tiamulin (TIA), tylosin (TY) and vancomycin (V).
Results: The percentage of these isolates for serotype 1, 2, 3, 7, 8, 9, 16 and untypable strains were 18%, 8%, 12%, 13%, 9%, 12%, 1% and 27%. Strains isolated the most from brain, lung and joint were serotype 7 (67%), serotype 3 (25%) and serotype 1 (50%), respectively. The percentage of antimicrobial agents resistance of these isolates for AMO, CZ, CT, DO, ENR, E, FFC, GN, L, LS, OTC, PG, TS, TIA, TY and V were
3%、92%、2%、53%、47%、87%、24%、32%、100%、67%、96%、36%、4%、60%、95% and 3%, respectively.
Conclusion: Compared to serotype 2 predominant in China and Canada, serotype 1 is the major serotype in Taiwan. It seems that serotype 1 have gradually increased in Taiwan which differed from China and Canada. Also, serotype 1, the predominant serotype in Taiwan, was not the mainly serotype isolated from brain and lung. It may related to specific virulence factors, need for further more study. About the antimicrobial resistance, *S. suis* was more sensitive to amoxicillin, ceftiofur and sulfamethoxazole-trimethoprim. In the contrast, it was resistance to cefazolin, lincomycin, oxytetracycline and tylosin. Therefore, clinical veterinarian can use the sensitive drug mentioned above to reduce the occurrence of antimicrobial resistance.

Disclosure of Interest: None Declared

Keywords: Antimicrobial resistance, Serotyping, Streptococcus suis

Bacteriology and Bacterial Diseases
S.SUIS

PO-PF3-130

Long term monitoring of susceptibility of Streptococcus suis isolates to amoxicillin from clinical cases on Czech swine farms

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Introduction: *S. suis* is recognised as major swine pathogen associated with the intensification of the swine industry worldwide. Until now limited number of effective vaccines are available. Therefore antimicrobial treatment is considered as one of the most effective approach for the control of streptococcal meningitis and polyarthritis.. Beta-lactam antibiotics and especially amoxicillin is considered as drug of first choice based on several criteria such as the PK/PD parameters and susceptibility (MIC) patterns. Aim of this study was long term amoxicillin susceptibility monitoring in clinical isolates of *S. suis* from Czech farms
Materials and Methods: 50 clinical isolates of *S. suis* serotype 1 and 2 were chosen in to the study. Isolate was represented one herd per year from the widest random coverage in the Czech Republic in the period 2005-2012. Isolates were obtained from clinical cases of meningitis and or arthritis) in suckling piglets and growers. Identification of *S. suis* isolates was performed based on biochemicaltests (API20Strep, BioMerieux, France) and confirmed by MALDI TOF MS (Bruker). Serotypes 1 and 2, as well as genes responsible for virulence were identified by specific primers using PCR. The MICs were determined using the agar macro-dilution method on Mueller-Hinton agar (Oxoid, UK), with the addition of 5 % sheep blood according to CLSI guidelines (2013), The range of amoxicillin concentration was ≤ 0,015 to &- mg/L. Two independent examinations for each strain were made.
Results: Because interpretative criteria for the susceptibility testing of *S. suis* of porcine origin have not yet been determined, we have used following criteria for streptococci (viridans group) CLSI M31-A3 (CLSI, 2013): category sensitive ≤ 0.12 mg/l, intermediate 0.25 to 2mg/l, resistant ≥ 4,0 mg/l. Using these criteria 5 (10%) strains out of 50 were recognised as intermediately sensitive to amoxicillin and no resistant isolate detected. Both parameters MIC 50 = ≤0,015 mg/l and MIC 90 0,125mg/l belongs to sensitive pattern.
Conclusion: Results obtained in this study demonstrate that pathogenic strains of *S. suis* isolated from pigs with streptococcal meningitis and arthritis in Czech Republic remain highly susceptible to amoxicillin and there is no evidence for resistance during 7 years period. We can conclude that amoxicillin remains as very effective drug for treatment approach against Czech *S. suis* isolates within *in vitro* susceptibility testing which is confirmed by clinical outcome of treatment and is in alignment with other similar studies worldwide.

Disclosure of Interest: None Declared

Keywords: antimicrobial resistance, streptococcus suis

Poster Abstracts

Bacteriology and Bacterial Diseases
S.SUIS

PO-PF3-060

An acute outbreak of Streptococcus (S.) suis in an integrated high health herd with heat stress as a possible trigger

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Introduction: The report describes a sudden outbreak of disease in suckling piglets associated to *S. suis* in a high health herd where *S. suis* previously never had been demonstrated.
Materials and Methods: The high health herd was established by purchase from two SPF herds in 2002-2003, and had since been self-recruting. New genes were introduced by AI. The farm was declared free from PRRS, APP, SEP, AD, SIV, *B. hyodysenteriae, Salmonella* spp. and *S. scabei* and comprised of 324 sows in 6 groups with batch-wise farrowing, all in-all out with washing and desinfection beetween batches in farrowing and nursery.
Results: On the 3rd of July 2015 the maximal outside temperature was 32 C and temperatures in the farrowing unit reached above 30 C. This had a negative effect on the pen hygiene and several sows refused feed for one or a few days.
During the 6th- 7th of July, an increased number of piglets aged 1-2 weeks were diagnosed with arthritis in a group with 54 litters, and a majority of the litters were affected. Clinical signs included high fever, severe lameness from one or more joints, fatigue and mild CNS syptoms. In most cases several piglets in a litter were ill, and in some litters all piglets were affected. In total, 150 piglets were treated individually with injections of long-acting procaine benzyl penicillin. Despite this the mortality increased in affected litters. The sows dis not express any signs of clinical disease.
A farm visit was made on the 8th of July. Three acutely ill piglets were euthanized and samples were collected from affected joints. *S. suis* was demonstrated by cultivation in affected joints from two of the piglets. Following that diagnose, all remaining piglets were treated individually with injections of long-acting procaine benzyl penicillin at the 13th and 15th of July.
The total mortality attributed to the outbreak amounted to 110-120 pigs. Only a few piglets died after the 15th of July.
Conclusion: No previous signs of infections with *S. suis* had been noticed in the herd that had been established from other SPF herds 13 years earlier and closed ever since. Still, as no way of introduction was identified, the most probable explanation for the outbreak is that the infection was latent in the herd, and was activated by the heat stress and the subsequent imparied hygiene. S. suis has not been diagnosed in the herd after this incidence.

Disclosure of Interest: None Declared

Keywords: heat stress, SPF herd, streptococcus suis

Bacteriology and Bacterial Diseases
S.SUIS

PO-PF3-074

Streptococcus suis antimicrobial susceptibility: A comparison of clinical isolates from pigs in England in 2010-2011 and 2013-2014

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Introduction:
Reports of increased antimicrobial resistance (AMR) in *Streptococcus suis* over time have been made in the literature. The aim of this study was to evaluate antimicrobial susceptibility in *S.suis* isolates from clinical cases of *S.suis* disease in pigs in England collected in two different time periods at each end of a five-year interval.
Materials and Methods:
A total of 210 clinical *S.suis* isolates from systemic sites or lungs from pigs with pneumonia from *S.suis* disease-related cases submitted to the Animal and Plant Health Agency (APHA) in 2010-2011 (n=93) and 2013-2014 (n=117) were examined. Minimum inhibitory concentrations (MICs) for 17 antimicrobials (amoxicillin, amoxicillin/clavulanic, penicillin, ceftiofur, cefquinome, doxycycline, tetracycline, tiamulin, sulphamethoxazole/trimethoprim, enrofloxacin, marbofloxacin, tilmicosin, tylosin, erythromycin, lincomycin, spectinomycin and florfenicol) were established by broth microdilution method according to Clinical & Laboratory Standards Institute (CLSI) guidelines. Results were analyzed as follows:

1. Classification as resistant or susceptible based on available CLSI clinical breakpoints.
2. Classification as wild-type (WT) or non-wild type (NWT) based on epidemiological cut-off values: WT phenotype below cut-off and NWT above cut-off.
3. MIC value distribution.
4. Prevalence of isolates classified as NWT for multiple antimicrobials.

Results:
The prevalence of AMR was higher in 2013-2014 than 2010-2011 for 7/17 antimicrobials tested. Four features were notable:

1. The prevalence of resistance was significantly increased for tetracycline and for sulphamethoxazole/trimethoprim based on available CLSI clinical breakpoints.
2. The prevalence of NWT was significantly increased for marbofloxacin, sulphamethoxazole/trimethoprim and spectinomycin.
3. There was a trend for increased MIC (also known as “MIC creep”) in 2013-2014 compared to 2010-2011 for ceftiofur, cefquinome, and also within NWT populations for tetracycline and doxycycline.
4. A significantly increased prevalence of isolates classified as NWT to multiple antimicrobials between both periods.

Conclusion:
Monitoring changes in antimicrobial susceptibility through assessing **MICs, NWT prevalence and MIC creep** can help **evaluate** the potential for development of clinical antimicrobial resistance.
The data adds to that available through other AMR surveillance in England and Wales and is **valuable information for veterinarians** medicating pigs for *S.suis* infections.

Disclosure of Interest: J. Hernandez-Garcia Conflict with: Zoetis, Conflict with: University of Cambridge, J. Wang: None Declared, T. M. Wileman Conflict with: Zoetis, Conflict with: University of Cambridge, O. J. Oshota: None Declared, L. A. Weinert: None Declared, S. E. Peters: None Declared, O. Restif: None Declared, M. A. Holmes: None Declared, S. M. Williamson: None Declared, D. J. Maskell: None Declared, A. W. Tucker: None Declared

Keywords: Antimicrobial susceptibility, MIC, Streptococcus suis

Bacteriology and Bacterial Diseases
S.SUIS

PO-PF3-001

Differences in antimicrobial susceptibility profiles among Streptococcus suis isolates from clinically and non-clinically affected pigs.

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Introduction:
Current information places the number of St*reptococcus suis* (*S.suis*) serotypes at between 29 and 33. Although only a subset of these is typically associated with most systemic disease, the upper respiratory tract (URT) of most pigs is colonised with non-clinical isolates. Recent studies described genomic differences between clinical systemic-located isolates and non-clinical isolates from URT. This study evaluated differences in antimicrobial resistance between these two types of isolate from clinically and non-clinically affected pigs.

Materials and Methods:
Ninety three clinical and 66 non-clinical *S.suis* isolates from pigs submitted to Animal and Plant Health Agency in England and Wales during 2010-2011 were examined. Clinical isolates (CI) were obtained from systemic samples or lungs of pigs with pneumonia. Non-clinical isolates (NCI) were from the URT of pigs without systemic disease or pneumonia due to *S. suis*.
The minimum inhibitory concentrations (MIC) of the isolates for 17 antimicrobials (amoxicillin, amoxicillin/clavulanic, penicillin, ceftiofur, cefquinome, doxycycline, tetracycline, tiamulin, sulphamethoxazole/trimethoprim, enrofloxacin, marbofloxacin, tilmicosin, tylosin, erythromycin, lincomycin, spectinomycin and florfenicol) were established by broth microdilution according to the Clinical & Laboratory Standards Institute (CLSI) guidelines.
MIC distributions (Mann-Whitney-Wilcoxon test), clinical breakpoints from the CLSI and epidemiological cut-off values (Pearson chi-square test) were analysed.

Results:
Percentages of tetracycline-resistant isolates were significantly different ($P<0.01$) for CI (77%) and NCI (97%). Percentages of isolates with MIC values over the epidemiological cut-off value were significantly higher ($P<0.01$) for NCI than CI for sulphamethoxazole/trimethoprim (20% vs 48%), tiamulin (11% vs 33%) and penicillin (12% vs 29%). Differences in MIC value distributions were also found for ceftiofur and cefquinome ($P<0.05$).

Conclusion:
This study found **reduced antimicrobial susceptibility among non-clinical versus clinical *S.suis* isolates** collected during the same time period. **Monitoring non-clinical isolates for antimicrobial susceptibility could therefore result in bias** and the results cannot necessarily be extrapolated to clinical isolates. Exchange of genetic material between clinical and non-clinical *S.suis* is possible, and **NCI could represent a reservoir of resistance genes**, constituting a potential threat.
Understanding the drivers and implications of relatively enhanced resistance in NCI of *S.suis* would be worthwhile.

Disclosure of Interest: J. Hernandez-Garcia Conflict with: Zoetis, Conflict with: University of Cambridge, J. Wang: None Declared, T. M. Wileman Conflict with: Zoetis, Conflict with: University of Cambridge, O. J. Oshota: None Declared, L. A. Weinert: None Declared, S. E. Peters: None Declared, O. Restif: None Declared, M. A. Holmes: None Declared, S. M. Williamson: None Declared, D. J. Maskell: None Declared, A. W. Tucker: None Declared

Keywords: Antimicrobial susceptibility, MIC, Streptococcus suis

Poster Abstracts

Bacteriology and Bacterial Diseases
S.SUIS

PO-PF3-175

Isolation rates of Streptococcus suis in carrier piglets after infection with H1N2

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Introduction: Secondary bacterial infections, especially with *Streptococcus (S.) suis,* are a leading cause of illness and death during influenza infection in pigs. Consequently, therapy of influenza A virus (SIV) infections using anti-inflammatory drugs is commonly completed with antibiotic treatment. However, the incidence of *S. suis* in SIV infected piglets and indications for antibiotic treatment have been scarcely investigated.
Materials and Methods: Thirty 10 weeks old piglets originating from a herd free of SIV and PRRSV, but with case histories of herd health problems caused by *S. suis* were divided into two groups. 15 piglets (group A) were infected intratracheally with H1N2 subtype A/swine/Kitzen/IDT6142/2007 ($15 \times 10^{7,25}$ $TCID_{50}$), while group B piglets were mock infected with PBS. Before infection, tonsillar swabs of all 30 piglets were taken and examined bacteriologically. Pigs were monitored daily for rectal temperature and clinical signs. On day 4, 6, 9, 12, 15, 42, 44 and 46 after inoculation two piglets of each group were euthanized and necropsied. Tonsillar swabs from all piglets as well as lung tissue samples and synovial swabs from piglets with patho-morphological findings were examined for *S. suis*. Recovered isolates were characterized by a multiplex PCR targeting the *S. suis* housekeeping gene *gdh* as well as capsular genes to differentiate serotypes 1, 2, 7 and 9. In addition, a PCR directed against genes coding for virulence-associated factors *epf*, *mrp*, *sly* and *arc*A was performed.
Results: Prior to infection, *S. suis* were isolated from the tonsils of 10 piglets of group A (67%) and 6 piglets of group B (40%), respectively. Further characterization of the *S. suis* isolates revealed only *arcA* positive but cps1, 2, 7 or 9 negative genotypes. While typical influenza clinical signs were observed after infection, no clinical signs characteristic for streptococcal disease occurred and *S. suis* detection rates on the tonsils were not increased. Some pigs tested positive before infection but became negative after infection and *vice versa*. After infection only from one tonsil a suilysin gene-positive *S. suis* strain was isolated. All lung and synovia samples were negative for *S. suis*.
Conclusion: In this trial no indication for antibiotic treatment during influenza infection could be deduced from the results. Tonsillar colonization of pigs with *S. suis* appeared to be independent of infection with SIV. Importantly, experimental SIV H1N2 infection of piglets carrying *S. suis* strains on their tonsils did not lead to detectable infection of the lung with *S. suis* or manifestation of a clinically apparent *S. suis* disease.

Disclosure of Interest: None Declared

Keywords: SIV, streptococcus suis

Bacteriology and Bacterial Diseases
S.SUIS

PO-PC02-003

Streptococcus suis colonization of newborn piglets on a farm with a history of streptococcal diseases

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Introduction: In an Austrian piglet producing farm with 120 sows and a history of streptococcal diseases in the nursery (meningitis, arthritis, sudden death), *Streptococcus (S.) suis* serotype 7 (*mrp+, sly-)* and *S. suis* of unknown serotypes (*mrp+, sly+, cps1-, cps2-, cps7-, cps9-*) were identified as etiological agents. Since many different attempts to minimize the problems failed, the objective of this study was to determine if *S. suis* colonization already occur during birth.
Materials and Methods: During the examination period no antibiotic treatment was performed. Tonsillar swab samples from 19 sows were examined during pregnancy. Vaginal swabs were taken during farrowing and in the case of vaginal discharge during the lactation period. In addition, tonsils and noses of six randomly chosen piglets per litter (n=114) were sampled immediately after birth before floor contact. All *S. suis* isolates recovered were further characterized by multiplex PCR targeting the housekeeping gene *gdh*, the capsular genes 1, 2, 7 and 9 and genes encoding virulence-associated factors (*epf*, *mrp*, *sly* and *arc*A).
Results: During pregnancy 63% of the sows were tested positive in their tonsils for *S. suis* serotype 7 (mrp-, sly-, epf-, arcA+) and 21% for *S. suis* of unknown serotypes (arcA+, *cps1-, cps2-, cps7-, cps9-).* Vaginal samples were tested positive for *S. suis* in 32 % of the sows, but none was positive for serotype 7. Nevertheless, seven piglets were carriers of serotype 7 (mrp-, sly-, epf-, arcA+) immediately after birth. Although born from sows tested negative in vaginal swabs, newborn piglets were in part positive for *S. suis* in their nose (15.2 %) and tonsils (11.4 %). In total, 21 % of the piglets were contaminated by *S. suis* intranatally. Only in three piglets, tonsils and nose were colonized at the same time. Piglets from the six sows tested positive in vaginal samples were either negative or positive for other *S. suis* strains immediately after birth. *S. suis* strains isolated in this study were different from those previously isolated from diseased animals.
Conclusion: Some piglets were infected during birth with *S. suis,* which was detected more frequently in nasal than in tonsillar swabs. Intranatal infection did not necessarily occur, even if sows were carriers. The transmission rate was very low. Since different *S. suis* strains were detected in pig groups and also in single individuals, further characterization of *S. suis* strains is necessary to get information about time of infection with strains responsible for herd problems.

Disclosure of Interest: None Declared

Keywords: None

Bacteriology and Bacterial Diseases
S.SUIS

PO-PF3-146

Analysis of genetic diversity and virulence of Streptococcus suis isolated from diseased pigs in Korea

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Introduction: *Streptococcus suis* is one of the most important pathogens in the porcine industry causing septicemia, meningitis and many other infections. Recently, Multilocus sequence typing (MLST), a highly discriminatory method used to characterize bacterial population structure, has been performed to investigate genotypes and microevolution of *S. suis* since 2002. Thus far, 1,467 *S. suis* strains have been recorded in the *S. suis* MLST database. However, their roles in the pathogenesis of human and swine infections remain poorly understood. Therefore, the purpose of this study is to analysis of genetic diversity and virulence of *S. suis* from diseased pigs in Korea.
Materials and Methods: A total of 27 *S. suis* strains isolated from pigs with presenting clinical signs of infection (arthritis, septicemia and pneumonia) were used in this study. Seven *S. suis* housekeeping genes were amplified for the 27 *S. suis* genomic DNA preparations using the primer sequences. MLST alleles of the individual genes, sequence types (STs) and clonal complexes (CC) were identified using the *S. suis* MLST database and eBURST Web application (http://ssuis.mlst.net/). The presence of *arcA*, *bay046*, *epf*, *hyl*, *mrp* and *sly* was determined by conventional and multiplex PCR.
Results: Twenty seven isolates could be assigned into serotypes 2 (7 isolates), 13 (10 isolates), 20 (3 isolate), 23 (3 isolate) and 31 (4 isolates). The 27 isolates could be assigned into 7 known STs [ST25 (2 isolate), ST27 (6 isolates), ST28 (1 isolate), ST87 (1 isolate), ST94 (1 isolate), ST108 (2 isolate) and ST117 (1 isolate)], and 5 novel STs [ST625 (1 isolate), ST626 (1 isolate), ST627 (1 isolate), ST628 (1 isolate), and ST630 (1 isolate). ST25 belonged to the ST25 complex, and ST27, ST28 and ST117 belonged to the ST28 clonal complex. All the 27 strains were subjected to PCR-based screening of six virulence markers, namely, *mrp*, *sly*, *epf*, *hyl*, *acrA*, and *bay046*. acrA and *bay046* were detected in all the 27 strains. *sly* and *hyl* were found in the novel ST strains (ST626 and ST630) but not in the other strains. *mrp* was found in only ST27.
Conclusion: In summary, Major serotypes of 27 *S. suis* strains isolated from diseased pigs in Korea. were 13, 2, 31, 20 and 23. Virulence genes were detected in all the 12 strains. *sly* and *hyl* were found in the novel ST strains (ST626 and ST630) but not in the other strains. *mrp* was found in only ST27. These results illustrated that the zoonotic strains of *S. suis* in Korea are continually evolving; therefore, increased surveillance of *S. suis* in farm-raised pigs should be conducted.

Disclosure of Interest: None Declared

Keywords: genetic diversity, Streptococcus suis, virulence

Poster Abstracts

Bacteriology and Bacterial Diseases
SALMONELLA

PO-PCO1-012

Vaccination with Enterisol® Salmonella T/C reduces Salmonella enterica colonization of ileocecal lymph nodes in growing pigs

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Introduction: Salmonellosis in pigs may have both production and food safety impacts. Vaccination has shown to improve pig performance and reduce colonization of pork carcasses. In this study, lymph node colonization was compared between a new, bivalent *Salmonella* vaccine (Protocol A) and a baseline vaccine program (Protocol B).
Materials and Methods: Pigs from a 5000 sow farm were placed into a *Salmonella* positive wean to finish barn containing 6 rooms, 288 pens and ~6600 pigs. Pigs were vaccinated in drinking water with Enterisol Salmonella T/C® (Boehringer Ingelheim, St Joseph, MO)(44 pens, 1100 pigs; Protocol A), or a baseline vaccination program (remaining pigs; Protocol B). All other medication, vaccination, feed, water, labor and management was applied uniformly. Pigs selected for harvest were placed by treatment onto two seperate semitrailers (one per group) and transported approximately 12 hours for harvest. Oral fluid samples were collected from pigs at loading (3/protocol), the common loading chute (1/chute) and transport trailers (8/trailer) prior to loading, and larriage pens prior to pig arrival (four/pen; 1/alleyway). Pigs were harvested by protocol, each after a production break and cleaning of facilities. Protocol B pigs were harvested ~two hours after arrival; protocol A pigs were harvested two hours later. Blood (171 A, 166 B) was collected after stunning, lymph nodes (153 A,137 B) after removal of viscera sets, and chilled diaphragm (172 A, 171 B) and carcass swabs (342; Speci-sponge) the following day. Serum, swabs and meat were sent on ice overnight for ELISA or qualitative culture. Lymph nodes were frozen and held for culture. Serum and meat juice samples were tested for antibodies against *Salmonella enterica* by ELISA (SVANOVA, Sweden). Swabs and lymph nodes (0.1g amounts) were cultured via selective enrichment at ISU-VDL(Ames, IA). Data was analyzed using JMP 3.0 (SAS Institute, Cary, NC).
Results: All farm pre-load samples (0/6) and transport (0/16) samples were culture negative. All lairage samples (9/9) and one carcass swab were positive. Protocol A pigs were positive for *Salmonella enterica* at a significantly lower rate than protocol B pigs (12% vs 63%, $p<0.0001$). Protocol A pigs had a higher seroprevalence rate in meat juice (75% vs 42%, $p<0.001$) but not serum (98% vs 95%, $p=0.26$). There was a two log reduction in CFU in protocol A vaccinated vs protocol B lymph nodes (median= 10^3 vs 10^5, respectively).
Conclusion: Vaccination with Enterisol Salmonella T/C® signficantly reduced colonization of pigs with *Salmonella enterica* species compared to baseline control vaccination. It should be considered to improve clinical control and reduce carriage in lymph nodes at harvest.

Disclosure of Interest: None Declared

Keywords: Colonization, Enterisol Salmonella T/C, Ileocecal lymph nodes

Bacteriology and Bacterial Diseases
SALMONELLA

PO-PW1-273

Isolation of lytic phages for Salmonella sp. wastewater from industrial pig farms in Chile.

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Introduction: *Salmonella* is a prevalent pathogen agent in the global pork industry and constitutes a serious risk to public health because it can contaminate meat and pig meat products. Bacteriophages are the most abundant biological entity on the planet and are able to alter the diversity and characteristics of bacteria via several mechanisms, being very interesting its lytic capacity. The aim of this study was to isolate and characterize *Salmonella* specific phages, capable of lysing predominant *Salmonella* in industrial pig farms in Chile.
Materials and Methods: During the months of January to October 2015, wastewater samples from 10 fattening pig farm were collected. These samples were cultured (1:10) for 24 hours at 37 °C in Trypticase soy broth (TSB) supplemented with 4 *Salmonella* serotypes (Infantis, Heidelberg, Typhimurium and Enteritidis). Subsequently, the enriched samples were subjected to two-step filtrations on 0,45 µm and 0,22 µm. This was follow by a mixture of the filtrate, a 1:10 dilution of an overnight culture of the serotypes listed above, and 0,7% TSA (softagar), these mixtures were poured on TSA plates and were incubated at 37 °C for 12-18 hours. We characterized, (i) the presence, shape and diameter (mm) of the plaque-forming units (PFU), (ii) the host were these phages were isolated, and (iii) the origin of the samples.
Results: We isolated 20 *Salmonella* lytic phages, these represented phages from 7 farms (70%), 9 (45%) of which were obtained on *S.* Enteritidis, 6 (30%) on *S.* Heidelberg, 4 (20%) on *S.* Typhimurium. In the case of *S.* Infantis, only one (5%) phage was observed. On two pig farms we obtained phages on strains representing serotypes *S.* Heidelberg, *S.* Typhimurium and *S.* Enteritidis. On 3 farms we obtained phages on 2 different combination of *Salmonellas* serotypes; we identified, one farm with *S.* Typhimurium and *S.* Enteritidis phages; one farm with *S.* Heidelberg and *S.* Typhimurium phages, and one farm with *S.* Heidelberg and *S.* Enteritidis phages. In the other 2 pig farms only *S.* Enteritidis allowed for phage isolation. Regarding the size of phage plaques, we observed diameters ranging from 1 mm to 5 mm, this indicating a variety of patterns lysis.
Conclusion: Our results show that the lytic phages for *Salmonella* are widely distributed in Chilean pig farms, this could indicate, either the presence of *Salmonella* in the farms, or a role in reducing *Salmonella* by phage populations. This is the first study of this kind in Chile, a collection of lytic phages isolated here could be used to reduce *Salmonella* in industrial pig farms and pork meat.

Disclosure of Interest: None Declared

Keywords: bacteriophages

Bacteriology and Bacterial Diseases
SALMONELLA

PO-PW1-272

Efficacy of different disinfectants intended for a pig farm environment.

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Introduction: Disinfection is a widely accepted element of disease control, although there are many types of product, with differing chemistries, which affects their activity against pathogens such as *Salmonella*.
This study investigated the ability of fifteen disinfectants to eliminate pig-associated *Salmonella*, specifically focusing on monophasic *Salmonella* Typhimurium (*S.* 4,5,12:i:-). The study included three m-cresol, one glutaraldehyde/ formaldehyde, four glutaraldehyde/quaternary ammonium compounds (QAC), two iodine, two peracetic acid and three potassium peroxomonosulphate-based commercial disinfectants.
Materials and Methods: Eight *Salmonella* serovars; *S.* Typhimurium DT193, two *S.* 4,5,12:i:- with different resistance profiles, *S.* 4,12:i:-, *S.* Derby, *S.* Bovismorbificans, *S.* Kedougou and *S.* Panama, isolated from pigs, were screened against all products using minimum inhibitory and minimum bactericidal concentration testing. There were no significant differences in MIC or MBC values between the serovars.
One *S.* 4,5,12:i:- DT193 strain , resistant to ampicillin, streptomycin and compound sulphonamides, was selected for further testing due to its relevance to recent cases of human s*almonellosis*. All fifteen products were diluted at the Department for Environment, Food and Rural Affairs (Defra) Approved Disinfectant General Orders (GO) concentration, half GO and twice GO, in World health Organisation (WHO) standard hard water.
Results: The disinfectants were tested using a faecal suspension model, a surface contamination model and a biofilm model to replicate boot dip and animal house cleaning disinfection. All products eliminated *Salmonella* in the boot dip model, the majority of the time at GO concentration. Only one glutaraldehyde/QAC-based product and one glutaraldehyde/formaldehyde-based product eliminated *Salmonella* when tested using the surface contamination model at GO concentration. The most successful products to eliminate *Salmonella* from biofilms were one m-cresol and the glutaraldehyde/formaldehyde based products, however products within the same chemical group demonstrated differing levels of effectiveness; again the presence of faecal material had a negative impact.
Conclusion: The type of product chosen can impact on the efficacy of farm disinfection; therefore, clearer guidance is needed to ensure the appropriate product is being used in order to control disease.

Disclosure of Interest: None Declared

Keywords: biosecurity, Disinfectants, farmed pigs

Bacteriology and Bacterial Diseases
SALMONELLA

PO-PW1-270

Eradication of Salmonella typhimurium DT 104 from a Swedish Multisite pig production system

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Introduction: The prevalence of Salmonella is very low in Sweden. Salmonella is included in the Zoonotic Act and controlled from farm to fork. Management of outbreaks on herd level focus on eradication with great demands on leadership, structure, systematic follow up and psychology. The report describes eradication of Salmonella from a large farrow to finish (FTF) herd.
Materials and Methods: In January 2012 one sow from a FTF herd with 850 sows was confirmed positive for Salmonella typhimurium DT104 (DT104) in the abattoir surveillance program. The herd had 3 barns with dry sows and 6 farrowing units, one barn with 6 weaner units, one barn with 4 weaner units and 2 units for replacers, and totally 4 fattening barns with 20 units.
A group that included the farmer, the herd veterinarians, SJV (the Board of Agriculture) and SVA (the National Veterinary Institute) led the eradication attempt. Maps of all buildings were made and all animal movements were documented. Hygienic zones were immediately established and a new clean mating unit was created. The herd was continuously visited and supported during the process.
Initially, every single pen at all parts of the farm, as well as the feeding system, were tested. Thereafter repeated strategical tests were made. Negative pigs were moved to clean units. Contagious pig were removed to decrease pathogen load and to create space for hygienic measures in a dynamic process where the hygiene zones altered with time. The farmer collected information of extraordinary measures daily to get correct refunds from SJV
Results: The feed was probably not the source of infection.
No DT104 was found in 3 out of the 4 fattening barns.
In one farrowing unit with 400 sows, DT104 was found in 10 dry sows that were euthanized. The remaining sows in this barn was moved to clean facilities.
DT104 was commonly diagnosed in the other barn with adults and piglets/weaners. All positive pigs were euthanized.
One piglet producing site was declared free from DT104 at the 10th of April and the other at the 23rd of July in 2012. The whole multisite production system was declared free from Salmonella at the 10th of August 2012. In total, 299 sows, 3 boars, 743 piglets, 1549 weaners and 179 fatteners were euthanized during the process.
Conclusion: By combining strict hygienic measures and proper management Salmonella can be eliminated from pig herds regardless of size, provided that all in/all out procedures are followed and the feed is free from salmonella.
To euthanize infected pigs was a dramatic step, but essential to create space in the system and allow disinfection of contaminated units.

Disclosure of Interest: None Declared

Keywords: None

Poster Abstracts

Bacteriology and Bacterial Diseases
SALMONELLA

PO-PW1-279

Comparative study of the occurrence of S. enterica serovar choleraesuis isolated from swine salmonellosis outbreaks during 2013 to 2015 in Brazil

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Introduction: *Salmonella enterica* serovar Choleraesuis has been identified in pigs worldwide. In Brazil, outbreaks with respiratory and circulatory disorders were observed by our laboratory during the year of 2013. In 2013, the pathogen was isolated from 28 production system (PS) originated from nine Brazilian states. *In vitro* susceptibility showed that the vast majority of the isolates (75%) were susceptible to lincomycin and spectinomycin. No information regarding the prevalence of this pathogen during the year of 2014 and 2015 in Brazil was described. The objective of this study is to evaluate and compare the occurrence of *S. enterica* serovar Choleraesuis in Brazil during the years of 2013 to 2015 and *in vitro* antimicrobial susceptibility.
Materials and Methods: Tissue samples submitted to our laboratory from 2013 to 2015 and positive for *S. enterica* serovar Choleraesuis were included in this study. If a PS had a single positive sample, the PS was considered as positive, regardless of the number of positive samples at the PS. One isolate from each PS, totalizing 64 strains, were selected to establish the *in vitro* susceptibilities against commonly used antimicrobials using Kirby-Bauer disk diffusion method. Each isolate was categorized as susceptible, intermediate and resistant for each drugs.
Results: During the year of 2013, 28 PS were considered positive for *S. enterica* serovar Choleraesuis based on at least one positive culture during the year. In 2014, 12 PS were positive for this pathogen and 24 PS were positive in 2015. Most of the PS had several positive samples. Antimicrobial susceptibility test revealed high frequency of resistance to Ampicillin, Amoxicillin and Tetracycline and high frequency of susceptibility to Linco/Spectinomycin.
Conclusion: The number of PS positive for *S. enterica* serovar Choleraesuis increased over the years in Brazil based on our laboratory results. This is likely due to a higher number of PS that submitted samples for diagnostic test over the years. Biosecurity measurements were implemented at one PS after an outbreak observed in 2013, confirming the importance of those measurements in controlling the disease. In conclusion, *S. enterica* serovar Choleraesuis continues to be an important pathogen in swine production in Brazil causing economic losses to the producer. The number of PS positive for the pathogen has increased likely due to a higher demand in diagnostic tests that occurred in our laboratory. The antimicrobial susceptibility of this pathogen has not changed over those 3 years.

Disclosure of Interest: None Declared

Keywords: Brazil, Linco/Spectinomycin, Salmonella enterica serovar choleraesuis

Bacteriology and Bacterial Diseases
SALMONELLA

PO-PW1-275

Experiences in the field of reducing the Salmonella burden on slaughter pigs in North Rhine-Westphalia, Germany

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Introduction: Since introduction of the regulation for reduction of *Salmonella* distribution of slaughter pigs in Germany in 2007, farmersare committed to analyse a defined sampling size of their pigs on a random basis of antibodies against *Salmonella*. Depending on the results, farms are classified in 3 categories (Category 1: 0-20% positive samples, cat. 2: 20-40% positive samples, cat.3 >40% positive samples).Farms with cat.3 are legally bound to search for sources of *Salmonella* and take action for reducing pathogen income. Therefore, bacteriological examinations have to been arranged and cleaning and disinfection measures as well as rodent control have to be improved.
Materials and Methods: In the period from 2012 to 2014 a total of 347 farms with *Salmonella* problems are visited by the animal health services North Rhine-Westphalia. Of these, 8 farms were rated in cat. 1, 94 in cat. 2, 141 in cat. 3. and 104 farms were without a category, since they were piglet producers or not classified yet. To find the sources of *Salmonella* a targeted sampling was conducted. Firstly, the empty stable was sampled to control cleaning and disinfection management. Secondly, pigs are sampled directly as soon as they are placed. Further sources of *Salmonella* income, like food, faeces of cats/ dogs or rodents were checked and sampled. Sampling materials were faeces (n=2145), blood (n=5356) and environmental samples (n=1047).
Results: In the bacteriological examination 20% of the faeces samples and 10% of the environmental samples were *Salmonella* positive. Interestingly, there were no positive environmental probes in cat. 1 farms, whereas 13% of these have been positive in cat.3 farms. Additionally, 18% of all blood samples had shown antibodies (titre> 15) against *Salmonella*.
Conclusion: Successful reduction of *Salmonella* is a result of complex control strategies. Since *Salmonella* is present in all stages of production, by our experience it's necessary to include the previous production stages (pig producers or even gilts) in the examination if a fattener gets *Salmonella* burdened pigs. Our main recommendations are based on three basic pillars: firstly by enhancing biosecurity to prevent *Salmonella* income, e.g. use of different clothes for each stable. Second step is performing a rigorously cleaning and disinfection management to avoid further distribution of the pathogens and thirdly is strengthening resistance of the pigs by reducing stress and dietetic measures, e.g. by supplementing acids to the feed ration.

Disclosure of Interest: None Declared

Keywords: control strategies, field co, Salmonella

Bacteriology and Bacterial Diseases
SALMONELLA

PO-PW1-268

Does the faecal microbiome of pigs affect Salmonella infection and shedding?

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Introduction: The microbiota of the porcine intestinal tract comprise a complex ecosystem which inhabit the gut from birth and provide many benefits to the host; including contributing to digestion, production of nutrients, development of the immune system and protection against pathogens. It is possible that variability in the level of colonisation and shedding of *Salmonella* in pigs can be influenced, at least partially, by intestinal microbial populations. The objectives of this study were to investigate if the early natural infection of weaned pigs by *Salmonella* is influenced by the intestinal microbiota and to compare faecal microbial profiles among pigs that regularly shed, did not shed or intermittingly shed *Salmonella* during the grower stage.
Materials and Methods: Fifteen pigs were monitored on a commercial farm during the weaning period (28 to 56 days of life). During this period, faecal samples were taken five times directly from the rectum and processed for the detection and isolation of *Salmonella* (ISO 6579/2007). A sub-sample of faeces was frozen in dry ice at the beginning, middle and the end of the study for microbial profiling of the faecal microbiota at phylum, family and genus level by Illumina MiSeq high throughput 16S rDNA gene sequencing.
Results: Similar to previous studies of the porcine microbiota, the principal phyla detected were *Firmicutes* and *Bacteroidetes,* with *Prevotellaceae* and *Prevotella* the main family and genus detected, respectively. Comparison of faecal microbial diversity and relative abundance of bacterial groups within the faecal microbiota of non-shedders, single point shedders, intermittent shedders and persistent shedders, is ongoing to determine if *Salmonella* shedding is associated with a particular microbial profile. Furthermore, changes in the faecal microbiome associated with *Salmonella* infection are being investigated. Finally, production data is being analysed to determine if changes in intestinal microbiota and/or *Salmonella* infection influence growth of the animals.
Conclusion: Overall, high throughput sequencing of the intestinal microbiota is a powerful tool. The results of this study will provide initial data on whether this technology can contribute to the clarification of some of the unanswered questions regarding *Salmonella* infection in pigs, thereby providing new insights for its prevention and control.

Disclosure of Interest: None Declared

Keywords: Infection, Microbiota, Salmonella

Bacteriology and Bacterial Diseases
SALMONELLA

PO-PW1-269

Salmonella in breeding pigs: Shedding pattern and transmission of infection to progeny in farrow-to finish herds.

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Introduction: *Salmonella* is one of the major causes of foodborne disease in humans worldwide, with a significant proportion of cases attributed to pork consumption. Availability of considerable data on infection patterns, risk factors and control strategies in finisher pigs contrasts with a scarcity of information about the role of breeding pigs in the transmission of infection. Unlike many EU countries, the principal method of pig production in Ireland is farrow-to-finish. The aim of the present study was to evaluate *Salmonella* transmission from sow to progeny under Irish production conditions and to establish whether there is a common shedding pattern in breeding pigs.
Materials and Methods: A longitudinal study was conducted on five farrow-to-finish commercial pig farms, selected based on historically high *Salmonella* seroprevalence. In each herd two batches of sows were randomly selected at service and monitored throughout a production cycle. Individual faecal samples were taken once at service, three times in gestation and three times during farrowing. The farrowing room floor was swabbed on the fifth sampling and a pooled faecal sample was collected from the piglets on the 6th and 7th sampling. Environmental pen swabs were also taken in the grower and finisher houses at least twice to determine the *Salmonella* strains present. All samples were analysed for *Salmonella* using the ISO 6579:2007 method. Any confirmed *Salmonella* isolates were further typed and tested for antimicrobial resistance.
Results: The prevalence of *Salmonella* shedding was low; 5% of sows were shedding at service, 1.6% in gestation and 2.5% after farrowing. *Salmonella* was detected in 4% of piglet faeces in the second week post-farrowing and 5% in the fourth week. *Salmonella* was detected in the farrowing room floor swabs on only one farm (13%). *Salmonella* shedding was higher at service and after farrowing on this farm compared to other farms as was the level of *Salmonella* shedding in piglets, with 11/68 (16%) samples positive in week 2 and 12/68 (17%) in week 4 post-farrowing. Overall, 21% of the pen swabs taken at weaning were positive for *Salmonella*, with 12% of the grower house and 36% of the finisher house pen swabs *Salmonella*-positive.
Conclusion: The results of the present study suggest that *Salmonella* shedding in sows is low in Irish production systems. A consistent pattern of *Salmonella* excretion was not observed on the five farms studied and in all except one, transmission to the progeny during farrowing seems to be negligible. Typing of the isolates is ongoing to investigate links among *Salmonella* isolates obtained.

Disclosure of Interest: None Declared

Keywords: Breeding pigs, Salmonella

Poster Abstracts

Bacteriology and Bacterial Diseases
SALMONELLA

PO-PW1-271

The antimicrobial resistant characteristics of Salmonella in pigs

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Introduction: *Salmonella enterica* is an important zoonotic pathogen and distributed around the world. Antimicrobial is the best medicine to control the infection of bacteria, but the antimicrobial resistance becomes more and more serious and threatens people's life. To investigate the antimicrobial resistant characteristics of *Salmonella* in pigs, a total of 55 *Salmonella* isolates from different provinces of China were studied.
Materials and Methods: In the antimicrobial susceptibility test, the broth microdilution method, referring to the describtion in the Clinical and Laboratory Standards Institute(CLSI, 2015), was carried out with the following 12 antimicrobial agents: amoxicillin , ampicillin, ceftiofur, gentamicin, kanamycin, tetracycline, doxycycline, chloramphenicol, florfenicol, sulfisoxazole, trimethoprim and ciprofloxacin. The quality control strain was *Escherichia coli* ATCC 25922. In the experment of antimicrobial resistance genes, a total of 23 genes were detected by PCR. In the mutation study of the Quinolone resistance-determining regions (QRDR) , the *gyrA* and *parC* genes had been sequenced.
Results: More than 50% resistant rates were observed to 11 antimicrobials(except trimethoprim), and 7 antimicrobials' resistant rates were 80% above, the highest of resistant rates were amoxicillin, chloramphenicol and tetracycline with 89.09%. In the mutli-antimicrobial resistant analysis, 54 isolates were resistant to 5~11 antimicrobial agents differently. Resisting to 10 antimicrobial agents was the largest group with 17 isolates (30.91%), which had 11 diferent resistant patterns. 15 antimicrobial resistant genes were postive by PCR, and the highest postive incidence was *sul2* gene (78.43%), followed by *tetA* (72.55%) , *sul1*(60.78%) and so on. Two mutation sites were found in *gyrA* gene: one is at the 83th site from Ser to Tyr (13/25), Leu (1/25) or Phe (2/25), another one is from Asp to Asn(2/25) or Tyr(1/25) at the 87th site.There were also two mutation sites in *parC* gene as follows: from Ser to Ile at the 80th site(2/25), from Ala to Ser at the 129th site(2/25).
Conclusion: Firstly, many antimicrobial agents have high resistant rates and large resistant patterns become prevalent. Secondly, antimicrobial resistance genes had different postive incidences in the different isolates, *dfrA5*, *dfrA7*, *dfrA13*, *tetG*, bla_{PSE}, *qnrA*, *qnrS* and *aac(6')-Ib-cr* genes are negtive. At last, two amino acids have mutated in *gyrA* gene or *parC* gene, it's a rare mutation of *parC* gene at the 129th site.

Disclosure of Interest: None Declared

Keywords: Salmonella, antimicrobial resistant characteristics , pigs

Bacteriology and Bacterial Diseases
SALMONELLA

PO-PW1-274

Oral and intranasal immunization of attenuated Salmonella vaccine strain expressing recombinant pertactin antigen of Bordetella bronchiseptica

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Introduction: *Bordetella bronchiseptica* is a highly transmissible respiratory pathogen that is etiological agent of atrophic rhinitis and bronchopneumonia in pigs. The disease constitutes a major public health burden in the swine industry worldwide. Several studies have shown that pertactin is important for protective immunity against *B. bronchiseptica*. Pertactin protein has two repeated regions, and region 1 (P1) and 2 (P2) are identified and characterized as an immunodominant protective-epitope. In addition, live attenuated *Salmonella* strains can induce efficient mucosal and systemic immune responses. We compared the immunogenicity of oral and intranasal vaccination with the attenuated *Salmonella* Enteritidis (SE) vaccine strain expressing P1 and P2 domain antigens of *Bordetella bronchiseptica* in BALB/c mice.
Materials and Methods: For experimental animal grouping, 50 female BALB/c mice were randomly divided into ten groups of 5 mice each. Mice were immunized orally (1 X 10^9 CFU) or intranasally (1 X 10^5 CFU) with HID2132 (SE *ΔaroAΔompA*), HID2133 (HID2132 with pJYTH01 vector), HID2134 (HID2132 with pJYTH-P1P2a), HID2135 (HID2132 with pJYTH-P1P2b) strains and phosphate-buffered saline (PBS). After 14 days, all mice were boosted with the same dose. Serum, feces and bronchoalveolar lavage fluid (BALF) samples were collected on days 0, 14, 28 and 42. All samples were analyzed using an indirect enzyme-linked immunosorbent assay (ELISA). Statistical analyses were performed with GraphPad Prism 5 software.
Results: All immunized mice survived without any symptoms and clinical signs of disease during entire experiment period. Primary immunization with recombinant SE mutants (HID2134 and HID2135) vaccine given both oral and intranasal induced significant levels of serum anti-*Salmonella* or anti-rP1P2 immunoglobulin G (IgG) and IgA antibody responses. A similar trend was observed in BALF samples of inoculated mice.
Conclusion: Our results have shown that oral and intranasal inoculation of the SE mutant expressing P1 and P2 domain of the pertactin of *B. bronchiseptica* produced good immune responses against both SE and *B. bronchiseptica* in mice. We suggest that this mutant could be an effective vaccine candidate.

Disclosure of Interest: None Declared

Keywords: Bordetella bronchiseptica, Pertactin, Salmonella

Bacteriology and Bacterial Diseases
SALMONELLA

PO-PW1-265

Salmonella Typhimurium fecal shedding following Salmonella Choleraesuis-Typhimurium vaccination via drinking water and challenge four weeks later

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Introduction: *Salmonella enterica* serovar Typhimurium (*ST*) and *Salmonella* Choleraesuis are primary pathogens in swine. *ST* is a primary cause of enteritis and subclinical production losses in growing or finishing swine and contributes to environmental and carcass contamination. Due to the zoonotic potential, intervention programs for *ST* have been established attempting to reduce carcass contamination. The objective of this study was to evaluate *Salmonella* fecal shedding of pigs vaccinated with a commercial, avirulent live culture (ALC) Salmonella Choleraesuis-Typhimurium vaccine when challenged with virulent *ST*.
Materials and Methods: Eight litters of two-week-old pigs were blocked by litter and assigned to treatment groups, 3 pigs/litter each to a Vaccine group (n=24) and to a Placebo group (n=24). Both treatments, Vaccine (Enterisol®Salmonella T/C) and Placebo, were administered through the drinking water. Pigs were housed by treatment during the vaccination phase to avoid unintentional exposure of ALC vaccine to the Placebo group and were re-penned individually with treatments comingled in the same room for the challenge phase. Four weeks after treatment, all pigs were challenged intranasally with 2mL of virulent *ST*($4x10^8$ CFU/dose). Fecal samples were collected daily for 14 days post-challenge (DPC) then three times weekly until 84DPC. Fecal samples were tested via modified enrichment culture (lower detection limit ~4000CFU/gram). The number of positive samples/pig in the Vaccine group was compared to the Placebo group using mitigated fraction analysis.
Results: During the 12-week challenge phase, the mean number of positive samples/pig was 26.2(Placebo) and 15.9(Vaccine) which was a significant improvement: mitigated fraction=0.788 and lower confidence limit = 0.5152. All Placebo pigs were positive from 3DPC to 6DPC, and ≥78.3% of Placebo pigs continued to shed until 31DPC; then at least 47.8% of Placebo pigs shed from 33DPC to 49DPC. For Vaccine pigs, 82.6% were positive the day after challenge which steadily declined to ≤13.0% from 47DPC through the end of the study.
Conclusion: Enteric pathogens have sporadic fecal shedding patterns as was observed in this study. While expectations are not that vaccination will eliminate shedding, the Vaccine group had significantly reduced shedding within two to six weeks post-challenge exposure while several pigs in the Placebo group continually shed through the challenge phase. Preliminary data suggests that the use of a commercially available ALC Salmonella Choleraesuis-Typhimurium vaccine clinically reduces fecal shedding in pigs post-challenge. At the time of submission for publication, the USDA had not reviewed this information and has not approved a shedding claim.

Disclosure of Interest: None Declared

Keywords: Fecal Shedding, Typhimurium, Vaccination

Bacteriology and Bacterial Diseases
SALMONELLA

PO-PCO1-016

The Prevalence of Salmonella from Cheek Meat and Head Trim in a Pork Processing Plant in the United States

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Introduction: Pork head meat, cheek meat, lymph nodes, and other carcass by-products may become contaminated with *Salmonella* in pork slaughter facilities. In a preliminary survey, a large pork processing plant in the United States was sampled bimonthly from January to July of 2015 to determine the prevalence, seasonality, and serotype diversity of *Salmonella enterica* (SE) isolated from cheek meat and head trim of swine carcasses.
Materials and Methods: Each cheek meat and head trim collection period (January, March, May, July) consisted of 25 samples collected on a Monday a.m., 25 on Monday p.m., 25 on Tuesday a.m., and 25 on Tuesday p.m., for a total of 100 cheek meat and 100 head trim samples (total of 200 for each period, total of 800 for 4 periods). Tissues were cultured for SE by described procedures using restrictive media and enrichment techniques. SE isolates were serotyped by the National Veterinary Services Laboratories, Ames, IA, USA.
Results: The percentages of SE-positive samples were 19.8% for cheek meat and 19.5% for head trim. The following were the results of isolations from cheek meat and head trim, respectively. January: 30% and 33%; March: 24% and 24%; May: 14% and 5%; and July: 11% and 16%. Serotypes (19) included: Derby; Heidelberg; Senftenberg; Muenchen; Typhimurium var 5-; Brandenburg; 4,12:i-; Rough_O:gst; London; Infantis; Enteritidis; Westhampton; Alachua; Ohio; Bredeney; 4,[5],12:i-; Mbandaka; Rissen; and Anatum.
Conclusion: These preliminary data suggest that pork products from the head may have a relatively high carriage rate of SE which includes a diverse population of serotypes, and based on results to date, there appears to be an effect of season on the prevalence of SE in head and cheek meat.

Disclosure of Interest: None Declared

Keywords: pork cheek meat, prevalence, Salmonella

Poster Abstracts

Bacteriology and Bacterial Diseases
SALMONELLA

PO-PW1-277

Cephalosporin resistant Salmonella isolated from conventional pig farms under different medication regimes

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Introduction: *Salmonella* is the second food-borne pathogen causing infections in humans and animals worldwide. Spain, the second producer of pork products for human consumption in the EU, reported a prevalence of *Salmonella* at slaughter of 29.4% in 2012.
This study intends to investigate the presence of cephalosporin resistant (CR) *Salmonella* in fattening pigs, and evaluate the possible association between consumption of antimicrobials during the rearing period and occurrence of CR *Salmonella*.
Materials and Methods: A total of eight pig farms were identified. In each farm, 70 seven-day old piglets were spatially separated in control (n=30), and treated group (n=40). In four of these farms, the treated group was medicated with ceftiofur (Naxcel®, Zoetis), whereas in the other four, seven-day old piglets were medicated with tulathromycin (Draxxin®, Zoetis Spain S.L.U). Prior treatment, faecal samples were collected from all piglets and sows. Faecal samples were also collected on days two and seven post-treatment and before the pigs departed to the abattoir. Presence of *Salmonella* in faeces was detected. An extra-plate containing ceftriaxone (1mg/ml) was included to detect CR *Salmonella*. Isolation was performed according to ISO 6579:2002.
Results: 66 out of 2096 faecal samples were positive for *Salmonella* and only two were obtained from the sows. *Salmonella* isolates were recovered from five different farms. The most prevalent serotype was Rissen (58%), followed by Typhimurium monophasic (14%), Brandenburg and Panama (11%), Anatum (5%) and Derby (3%). Three *S.* Rissen (bla_{ctxm-1}, $bla_{ctxm-14}$) and one *S.* Anatum (bla_{ctxm-1}) were resistant to cephalosporins. However, three of the four CR *Salmonella* were recovered in the first visit prior taking medication. One extra isolate was recovered 48 hours post-treatment with ceftiofur from one animal previously positive for CR *Salmonella* before treatment. CR *Salmonella* could not be detected during the rest of the study period.
By the finishing time, all samples were negative for CR *Salmonella* in all farms This is a relevant observation since different antimicrobials were used in a prophylactic way during the nursery period, including amoxicillin. Moreover, it was not observed any increase in the percentage of samples positive for CR *Salmonella* 48 hours post-treatment within the group treated with ceftiofur.
Conclusion: A direct relation was not established between the use of ceftiofur and tulathromycin and emergence of CR *Salmonella*. Resistance to 3rd generation cephalosporins was observed in *Salmonell*a isolates recovered from seven-day-old piglets prior taking medication.

Disclosure of Interest: None Declared

Keywords: antimicrobial resistance, Cephalosporins, Salmonella

Bacteriology and Bacterial Diseases
SALMONELLA

PO-PW1-001

Salmonella ELISA Serology from Weaned Pigs as an Indicator of Salmonella Circulation in Breed to Wean Sites

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Introduction: Understanding prevalence of *Salmonella* in market pigs has become a priority within USDA. With this priority pork production systems have a greater interest in understanding *Salmonella* prevalence in market pigs and the relationship of this prevalence to breed-to-wean sites. In a European study, a 90% reduction in breeding herd *Salmonella* prevalence reduced market pig *Salmonella* prevalence in the lymph-node by 66%. The objective of this study was to evaluate the ability of an indirect *Salmonella* ELISA antibody test (Vetsign™, Svanova) to detect *Salmonella* positive weaned pigs (5 & 9 weeks of age) and determine if weaned pig *Salmonella* antibodies could predict *Salmonella* circulation at breed-to-wean sites.
Materials and Methods: At risk breed-to-wean sites within a large pork production system were identified. These sites ranged in inventory from 300 to 800 sows. *Salmonella* risk factors identified at the selected sites included: 1) Open gutter flush 2) Rodent feces observed in barn 3) Sanitation practices below expectations. Weaned pigs from these sites were generally of good health and meeting company expectations only occasional post weaning diarrhea was observed.
Twenty, 5-week-old pigs from 12 breed to wean sites were tagged and bled after placement into the nursery facility. If ≥6 (30%) of the pigs demonstrated *Salmonella* ELISA antibodies the prevalence was considered high. These same 20 pigs were then retested 4 weeks later (9 weeks of age). Decreasing *Salmonella* ELISA antibodies from these paired samples could then be interpreted as decreasing maternal antibodies. For this study, if ≤5 weaned pigs were ELISA positive to *Salmonella* then the *Salmonella* prevalence of the breed-to-wean site was considered to be low.
Results: Sites A,B,C,E and F had ≥ 30% *Salmonella* ELISA positive pigs and these ELISA titers decreased 4 weeks later in serial bled pigs (suggesting these antibodies were maternally derived). The ELISA titer rose at site D four weeks later in serial bled pigs (suggesting active exposure). At sites G-L the *Salmonella* ELISA positive pigs were <30%. Pigs at sites G-L were not retested.
Conclusion: *Salmonella* ELISA titers in 5 and 9 week-old pigs appear to be a predictor of *Salmonella* circulation of breed-to-wean sites. Further investigation is planned at the source sites from which the 5-week-old pigs demonstrating ≥30% positive findings originated. Envirobooties™ (Hardy Industries) will be utilized at these sites to culture and identify *Salmonella* species.

Disclosure of Interest: None Declared

Keywords: ELISA, EnviroBootie, Salmonella

Bacteriology and Bacterial Diseases
SALMONELLA

PO-PW1-266

Evaluating the use of the EnviroBootie to detect Salmonella at five breed-to-wean sites

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Introduction: The EnviroBootie™ (Hardy Industries) has been used to detect *Salmonella* enteritidis in poultry and *Salmonella* choleraesuis and typhimurium in swine. The objective of this study was to utilize the EnviroBootie™ to identify *Salmonella* groups at 5 breed to wean sites suspected as having elevated *Salmonella* prevalence.
Materials and Methods: The 5 suspect sites chosen for bootie evaluation had previously demonstrated >30% prevalence of *Salmonella* ELISA antibodies in 5 week old piglets originating from these sites. The suspect sites evaluated demonstrated *Salmonella* risk factors to include; poor sanitation practices, visible rodents and/or feces and open gutter flush systems. The control site was a suspected low prevalence farm following the same general pattern as the other sites visited. The control site was selected as the 5 week old seroconversion was <10% of pigs tested. Biosecurity practices upon entry at each site included; Tyvek coveralls, clean boots and plastic boot covers. Sanitized collection kits (12) utilized at each site included: 2 gloves and 2 EnviroBooties™ contained in individual whirl-pack bags (24 total booties/site). After securing the EnviroBooties™ (1 on each foot) the technician walked behind each row of sows (2 rows/alley) taking slow and deliberate steps. After walking to the end of each area to be sampled, the environmental booties were taken off and put back into its individual whirl-pack bags and sealed. This walk was repeated 12 times in each barn. Prior to leaving the site the 24 bootie samples were immediately placed on ice and overnight shipped to the Iowa State University Veterinary Diagnostic lab for *Salmonella* culture.
Results: The positive *Salmonella* samples were grouped as B, C1, E, Poly, or untypable. Group B *Salmonella* was found at every site, and Group C1, was found at all but one site. All sites contained more than one group of *Salmonella*. Positive results were identified in locations that appeared fecal material free, such as pens, hallways, and chutes.
Conclusion: The EnviroBooties™ picked up many positive samples at the 5 sites. Multiple *Salmonella* groups were identified and were widely diverse among the different sites. *Salmonella* species were evident in all environments but clinically relevant in weaned pigs where evidence of rodents was observed in the breed-to-wean environments. Observable signs of rodents served as a visual risk factor suggestive of *Salmonella* challenge. The EnviroBootie™ is a sensitive method for picking up *Salmonella* isolates in pig environments.

Disclosure of Interest: None Declared

Keywords: Choleraesuis, EnviroBootie, Typhimurium

Bacteriology and Bacterial Diseases
SALMONELLA

PO-PW1-278

Clinical Salmonella reduction with Clostridium butyricum (Miya-Gold® S)

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Introduction: The mortality evolution is described in a 7-30 kg weaner pig farm before and after probiotic supplementation. The farm had increasing incidence of nonspecific diarrhea not responding to antibiotic treatment with isolation of different *E. coli* serotype 141 and *Salmonella* 4,12:i:.
Materials and Methods: A farm producing 35.000 7-30 kg pigs from own sow SPF herd (MYC+APP serotype 12 positive) vaccinating for *Mycoplasma hyopneumoniae* and PCV-2 had a history of slightly elevated mortalities, 3,4% in Q4 2014, 4,0% in Q1 2015. A sudden increase in mortality in Q2 2015 to 10,5% was seen. 700 weaned piglets arrived each week to washed, dried out and disinfected sections. Pens were sprayed with hydrated lime, and manure pits emptied and treated with hydrated lime. The feed was not pelleted and supplemented with benzoic acid. The first 14 days zinc oxide at 2500 ppm was added to the feed. The water is supplemented with formic acid 1500 ppm through a dosatron. The pigs were all affected with severe enteritis the first 14 days after arrival. Laboratory reports and necropsies showed necrotizing typhlo-colitis. Antibiotic treatments carried out according to laboratory antibiograms showed little or no effect on enteritis and mortality. In week 28 (first week of July) Miya-Gold® S was supplemented at 2 kg/MT in the first feed (6,2 kg pigs), 1 kg/MT feed in the second feed, 0,5 kg/MT feed in the third feed.
Results: Weekly mortalities dropped dramatically from 12% in week 27 to 3,1% in week 28. The average mortality in Q3 2015 was 3,3%, and 3% in Q4 2015. 10% of the pigs still had clinical signs after arrival, and were put to hospital pens. Average daily gain increased from 402 g/day in Q2 to 428 g/day in Q3 and 442 g/day in Q4 2015 respectively.
Conclusion: Miya-Gold® S is a zootechnical feed additive based on spores of *Clostridium butyricum*. These spores are stable against heat treatment, pelleting, gastric acid, bile and enzymatic degradation. Vegetative bacteria in the ileum and colon of the pig, produce butyric and acetic acid thus stabilizing the microflora of the pig. This case describes a consistent stabilizing effect of Miya-Gold® S on gut health creating a less favorable environment for pathogenic *E. coli* and *Salmonella spp.* resulting in less mortality in pigs of 7-30 kg.

Disclosure of Interest: None Declared

Keywords: Clostridium butyricum, gut health, Salmonella

Poster Abstracts

Bacteriology and Bacterial Diseases
SALMONELLA

PO-PW1-280

Yeast Mannan rich faction (Actigen) Reduces Adherence of Salmonella to Porcine intestinal cell line.

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Introduction: In swine, Salmonella infections cause two separate problems: clinical salmonellosis and a clinically silent carrier state that may cause food borne disease if faecal contamination of pork occurs. Salmonella infection can occur by a number of different mechanisms including attachment and/or invasion of bacteria to the cells. Mannan oligosaccharides structurally resemble the receptor sites coating the intestinal epithelium to which intestinal pathogens like salmonella adhere, these oligosaccharides act as molecular decoys which can competitively inhibit adherence of pathogens to the intestinal epithelium. The objective of this study was to determine if a commercial mannan rich fraction extracted from yeast (Actigen) could reduce adherence of a number of salmonella strains to intestinal porcine epithelial cells (IPEC-J2) *in-vitro*.
Materials and Methods: Briefly, IPEC J2 cells $2*10^4$ were cultured on 6 well plates using CO_2 independent medium. The adhesion test consisted of 30 min incubation of the Actigen with the bacteria at room temperature mimicking the delay before chyme reaches the small intestine. Then, 500uL of this mixture were added to each well, followed by 30 min incubation (at 37°C and 5% of CO_2) with the cell monolayer. Then the wells were thoroughly washed three times with sterile PBS, in order to remove the non-adhered bacteria and the residue of the Actigen and harvested. The cell lysate, which consisted of bacteria which adhered to the cells and invaded the cells, was plated on plate count agar incubated at 37°C and after overnight culturing colonies were counted.
Results: Adhesion tests with two different strains of Salmonella were performed; S. Typhimurium and S. Dublin. Actigen shows a clear ability to reduce the number of salmonella cells which adhered to the IPEC-J2 cells. In the case of S. Typhimurium the adherent cells decreased from $1x10^6$ CFU in the control untreated IPEC cells to $3.2x10^5$ with the Actigen treatment this represents a significant reduction of 67% with $p< 0.05$. In the case of S. Dublin the adherent cells decreased from $8.6x10^6$ CFU in the control untreated IPEC cells to $9.8x10^5$ with the Actigen treatment this represents a significant reduction of 89% with $p< 0.05$.
Conclusion: These results indicate that yeast mannan rich fraction (Actigen) can act as anti adhesive agents against Salmonella adherence to intestinal cells. In addition this data suggests that inclusion of Actigen in the diets of pigs could support functional activity against salmonella infection.

Disclosure of Interest: None Declared

Keywords: Actigen, Porcine intestinal cells, Salmonella

Bacteriology and Bacterial Diseases
SALMONELLA

PO-PW1-264

Observation of Salmonella Seroprevalence of Commercial Non-Vaccinated Pig Flow in the Eastern United States

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Introduction: An association between *Salmonella* serology and prevalence of *Salmonella* bacteria post-harvest has been established. Pork producers may be able to help reduce the prevalence of Salmonella at slaughter through on-farm interventions. Efforts have been concentrated to reduce post-harvest Salmonella contamination, but a better grasp on the level of *Salmonella* exposure occurring pre-harvest is needed. In an effort to understand the complex population dynamics of *Salmonella* exposure, a cross sectional analysis was performed to collect data from various stages of production.
Materials and Methods: Five sow farm flows in northeastern USA were enrolled. All of the pigs were housed in modern commercial facilities. Pigs were not vaccinated for *Salmonella* and originated from unvaccinated sows. One flow (A) was classified as having no clinical history of scours or *Salmonella*. Four flows were selected due to sporadic observations of scours thru grow-out (B, C, D, & E) although *Salmonella* was rarely cultured. Serum was collected from 10 pigs at 4, 7, 12, 16, 20, 24, and 27 weeks of age (n=350). All laboratory testing was done at the Boehringer Ingelheim Vetmedica, Inc. Health Management Center (Ames, IA). Samples were tested using VETSIGN™ Salmonella-Ab ELISA (SvanovaR) which has been shown to accurately detect a broad spectrum of serotypes in serum.
Results: Antibodies to *Salmonella* were detected in 46.4% of the pigs sampled. Antibodies to *Salmonella* were detected in 40% of the samples from flow A, while all other flows ranged from 35.7 to 62.9% positive. Across all flows, 24-week-old pigs consistently had the highest prevalence of *Salmonella* positive samples with 84% having antibodies. Alternatively pigs at 4 weeks of age had the lowest prevalence with only 18% of samples *Salmonella* positive. Pigs at 7, 12, 16, and 20 weeks of age had *Salmonella* positive samples 34, 38, 56, and 46% of the time respectively.
Conclusion: *Salmonella* antibodies were present in all flows regardless of historical observations. In older pigs, there was a peak increase in *Salmonella* positive samples which is consistent with previous literature. This could provide a single time point to monitor prevalence of *Salmonella* pre-harvest. Serology can be used as an indicator of *Salmonella* exposure pre-harvest, but needs to be used in combination with fecal culture and post-harvest surveillance in order to determine current *Salmonella* prevalence and contamination prior to interventions. Further investigation is necessary to determine if on-farm intervention programs can impact *Salmonella* fecal shedding and consequently reduce the prevalence of *Salmonella* at slaughter.

Disclosure of Interest: None Declared

Keywords: Salmonella, serology, seroprevalence

Bacteriology and Bacterial Diseases
SALMONELLA

PO-PW1-267

Serological evaluation of vaccination against Salmonella Typhimurium with an attenuated vaccine in two farrow-to-finish farms

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Introduction: Pig herds are frequently infected with *Salmonella* Typhimurium. Infections are mostly subclinical and difficult to control with the currently available control measures. Vaccination might be effective to control *Salmonella* infections at farm level and hence be a promising tool to reduce the risk for human salmonellosis.
Materials and Methods: In this study, five different vaccination strategies (1. vaccination of sows; 2. vaccination of sows and piglets; 3. vaccination of sows and fatteners; 4. vaccination of piglets; 5. vaccination of fatteners) were tested and compared to a negative control group (group 6) in two Belgian farrow-to-finish farms. Seventy-two (72) sows of one batch were divided into six groups (12 sows/group) and the pigs were followed until slaughter. An attenuated vaccine (Salmoporc®, IDT Biologika) was applied twice, each time with an interval of three weeks. The vaccine was administered subcutaneously in sows and fatteners and orally in piglets. Blood samples were collected before and after vaccination to evaluate the serological response of vaccination. The sera were analyzed by ELISA and the sample-to-positive-ratios (S/P-ratio) were assessed.
Results: Prior to vaccination, the mean S/P-ratio of the sows in group 1-3 and the sows in group 4-6 did not differ (farm 1: 1.448 and 1.398, p=0.876 – farm 2: 1.046 and 0.669, p=0.312). Three days after farrowing, the mean S/P-ratio of the vaccinated sows (group 1-3) was significantly higher than the mean S/P-ratio of the non-vaccinated sows (group 4-6) (farm 1: 2.525 and 1.111, p<0.001 – farm 2: 2.313 and 0.493, p<0.001). At three days of age, piglets from vaccinated sows had a significantly higher mean S/P-ratio compared to piglets from non-vaccinated sows (farm 1: 2.674 and 1.333, p<0.001 – farm 2: 2.685 and 0.399, p<0.001). A high Pearson-correlation between the S/P-ratios of the sows and the S/P-ratios of the piglets was found on both farms (farm 1: 0.934 – farm 2: 0.953, p<0.001).
At slaughter age, the S/P-ratios of the vaccinated pigs (group 1-5) did not significantly differ from the S/P-ratios of the negative control group on farm 2. In farm 1, the S/P-ratios of the vaccinated fatteners (group 3 and 5, means: 2.130 and 3.489) were significantly higher compared to the S/P-ratios of the control group (mean: 1.240, p=0.041 and p=0.016).
Conclusion: Vaccination of sows induces a serological response and increases the degree of maternal immunity which is transferred from the sow to the piglets.
The high S/P-ratios of the vaccinated fatteners can be explained by the immune response induced by the vaccination and show that vaccination of fatteners might influence the results of serological monitoring programs currently in use.

Disclosure of Interest: None Declared

Keywords: Attenuated vaccine, Salmonella Typhimurium, Serological evaluation

Bacteriology and Bacterial Diseases
SALMONELLA

PO-PW1-276

Effect of seaweed extracts and galacto-oligosaccharides on growth performance and intestinal health in pigs following S. Typhimurium challenge

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Introduction: Pork and pork products are recognised as vehicles of *Salmonella* Typhimurium (*S.* Typhimurium) infection in humans. In recent years, galacto-oligosaccharides (GOS) and seaweed extracts (SWE) have been explored as novel sources of bioactive compounds that contain antimicrobial and immunomodulatory properties. The main bioactives in seaweed are the polysaccharides laminarin and fucoidan, which have antimicrobial, prebiotic and anti-inflammatory properties. Galacto-oligosaccharides are prebiotics and possess immunomodulatory properties, either through modification of intestinal microbiota and/or reducing the effects of intestinal inflammation. The objective of this study was to assess the effects of GOS and SWE on reducing *S.* Typhimurium numbers and intestinal inflammation *in vivo* after experimental challenge with *S.* Typhimurium.
Materials and Methods: Thirty pigs (n = 10/treatment, live weight 30.9 kg) were randomly assigned to 3 dietary treatments: (1) basal diet; (2) basal diet + 2.5 g/kg GOS; (3) basal diet + SWE (containing 180 mg/kg laminarin + 340 mg/kg fucoidan). Following an 11 day dietary adaptation period, pigs were orally challenged with 10^8 CFU/ml *S.* Typhimurium. Initial weight, weight at challenge and slaughter weight were recorded. In addition, feed intake was measured for the purpose of calculating average daily feed intake and feed efficiency. Fresh faecal samples were collected pre-challenge and on d 2, 4 and 7 post-challenge to enumerate *S.* Typhimurium. Pigs remained on their diets for a further 17 days and were then sacrificed. Digesta samples from the colon and caecum were collected for microbial analysis. Tissue was collected from the ileum and colon for cytokine gene profiling.
Results: Supplementation with SWE reduced *S.* Typhimurium numbers in faecal samples collected 7 days post-challenge and in digesta from the caecum and colon ($P < 0.05$). *Lactobacillus* numbers were increased in the caecum and colon after GOS supplementation ($P < 0.05$). In the colon, both GOS and SWE supplementation reduced mRNA expression levels of *IL-6, IL-22, TNF-α* and *Reg3-γ* ($P < 0.05$). The growth performance and feed efficiency was improved after supplementation with GOS and SWE ($P < 0.05$).
Conclusion: It can be concluded that addition of SWE reduced faecal and intestinal *S.* Typhimurium numbers compared to the basal diet, while GOS supplementation improved intestinal *Lactobacillus* numbers but did not affect *S.* Typhimurium numbers. Supplementation with GOS and SWE reduced the intestinal inflammation of pigs and improved their growth performance after the experimental *S.* Typhimurium challenge .

Disclosure of Interest: None Declared

Keywords: Galacto-oligosaccharides, Salmonella Typhimurium, Seaweed extract

Poster Abstracts

Bacteriology and Bacterial Diseases
SALMONELLA

PO-PCO1-019

Persistence of Salmonella in the environment and role of wild birds in the epidemiology of infection in an outdoor pig farm in the United Kingdom.

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Introduction: The prevalence of *Salmonella* infection in breeding pig farms in the United Kingdom (UK) was reported to be 52.2% in 2008 by EFSA. In the UK, approximately 40% of the pigs are bred outdoors. More effective control of wild fauna, especially for outdoor farms, could result in a lower prevalence of *Salmonella*. This study aimed to investigate the role of wild birds in the epidemiology of *Salmonella* in one outdoor pig farm and environmental *Salmonella* persistence once the farm was empty of pigs.
Materials and Methods: Three sampling visits, at monthly intervals, were made to an outdoor pig farm consisting of three fields. Two of the fields had been empty of pigs for 2 (field 1) and 3 years (field 2) respectively and the third was occupied by pigs during the first visit only (field 3). Pooled faeces from wild birds and other wild animals, soil and surface water samples were collected at each visit. Individual pig floor faeces were collected at the first visit only. *Salmonella* was isolated according to ISO6579 Annex D, and serotypes were determined for all isolates of the first and second visit according to the White-Kauffmann-Le Minor scheme.
Results: At the first visit, *S.* 4,5,12:i:− (mST), *S.* Rissen and *S.* Panama were isolated from pig faeces. mST was also isolated from a water puddle and a rabbit and fox dropping. *S.* Typhimurium was isolated from one bird dropping of the 27 collected (3.7%). At the second visit, mST and *S.* Rissen were isolated from soil and water puddle samples in fields 1 and 3. Of 76 wild bird droppings tested, 22 (28.9%) were *Salmonella* positive (mST, *S.* Typhimurium, *S.* Senftenberg and *S.* Rissen). At the third visit, *Salmonella* was isolated from soil and water puddle samples from all 3 fields. Of the 79 wild bird droppings tested, 35 were *Salmonella* positive (44.3%).
Conclusion: The results from this study suggest that pigs are the likely source of *Salmonella* in the pig farm environment, since the serotypes isolated are also commonly found in housed breeding pigs. *Salmonella* was found in soil and water samples in the field that had been empty for 3 years. The *Salmonella* serotypes found in wild bird droppings were the same as those found in pigs. Once the pigs had left the farm, the proportion of *Salmonella* - positive wild bird faeces increased. This could be due to the fact that the wild bird population accessing the fields increased considerably once the pigs had left the farm, because of the presence of leftover pig feed. This study suggests that *Salmonella* can persist in soil and water in outdoor pig farms and that wild bird populations are capable of recycling the infection and contributing to the persistence of *Salmonella* between batches of pigs.

Disclosure of Interest: None Declared

Keywords: Outdoor pig farm, Environment, Wild birds

Bacteriology and Bacterial Diseases
SALMONELLA

PO-PW1-282

Ultraviolet (UV) inactivation of Salmonella choleraesuis and Salmonella typhimurium in native porcine plasma

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Introduction: Spray dried plasma (SDP) is a common ingredient in post-weaning pig diets due to its beneficial effects on performance and survival. Although the manufacturing process of SDP has been demonstrated to be safe for a variety of high thermal resistant pathogens, the introduction of additional safety features will further enhance the robustness of the manufacturing process. Ultraviolet at 254 nm wavelength (UV-C) is a non-thermal process that disrupts cellular transcription and replication inactivating many microorganisms, including bacteria and viruses. Presently, a special UV-C designed process for large industrial volumes of turbid liquids (such as animal plasma) is now available. The objective of this study was to evaluate the effectiveness of the UV-C irradiation using a proprietary system (SurePure SP1) on survival of *Salmonella choleraesuis* (S. chol) and *Salmonella typhimurium* (S. typh) inoculated in liquid porcine plasma (PP).
Materials and Methods: 24 L of liquid PP, obtained from an industrial plasma producer, was divided in three sub-batches of 8 L. At time zero, 15 mL samples were obtained and served as negative control sample, before bacteria inoculation. A positive control sample was collected 5 min after the PP was inoculated with S chol or S typh and before UV exposure. Both of the inoculated bacteria were resistance to 500 µg streptomycin/mL. Each sub-batch was exposed to UV-C at 1500, 3000, 6000 and 9000 J/L. The SP1 system was designed to maintain a turbulent flow with a minimum velocity of 4000 L/h continually recirculating the plasma until the desired exposure was achieved. After UV exposure, samples were log diluted in peptone water and 0.1 mL inoculated in TSA plates containing streptomycin (500 µg/mL) to enumerate the S. typh and S. chol.
Results: Liquid plasma was inoculated with 6.28 ± 0.08 and 6.51 ± 0.04 Log_{10} cfu/mL for S. typh and S. chol respectively (mean ± SD). UV radiation on S. typh produced a viability reduction of 2.63, 3.89, 4.08 and 4.56 log_{10} at 1500, 3000, 6000 and 9000 J/L, respectively. Reduction of S. chol was slightly higher, 3.42, 3.95, 4.29 and 4.60 log_{10} at 1500, 3000, 6000 and 9000 J/L, respectively.
Conclusion: Overall results indicated a reduction of both S. typh and S. chol around 4 log titres by an UV dose of 3000J/L. These results indicate the usefulness of the UV treatment to inactivate streptomycin resistant Salmonella species in liquid plasma as an additional safety feature for the entire process to manufacture spray dried plasma.

Disclosure of Interest: E. Blázquez Conflict with: APC-EUROPE, Conflict with: APC EUROPE, J. Ródenas Conflict with: APC EUROPE, C. Rodríguez Conflict with: APC EUROPE, J. Segalés: None Declared, J. Pujols: None Declared, J. Polo Conflict with: APC-EUROPE

Keywords: Salmonella, spray dried plasma, Ultraviolet UV-C

Bacteriology and Bacterial Diseases
SALMONELLA

PO-PW1-281

Salmonellosis caused by Salmonella takoradi observed in weaned pigs in Czech Republic

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Introduction: Rare *Salmonella* serovar S. *takoradi* was usually detected in chickens and poultry slaughterhouses mostly in Asia.
Materials and Methods: The farm where this disease outbroke was a 200 sows commercial production system without SPF status. Stables for pigs were reconstructed only partially, with problematic ventilation and heat control and with semi-continuous operation. Mortality of the weaned pigs ranged from 2 to 3 %.
The outbreak of salmonellosis started at the beginning of March 2015 and mortality of weaners was increased by more than three times. Weaned piglets in good condition began to show the first signs of diarrhea and wasting approximately 7 days after weaning, even after amoxicillin medication.
Laboratory examination confirmed *Salmonella takoradi* with antibiotic resistance to amoxicillin and tetracyclines. Other laboratory tests did not reveal infection caused by another specific pathogen. Quality of feed was also analyzed, again with negative results.
Based on established sensitivity to antibiotics, medication after weaning was switched to trimethoprim/sulphamethoxazole. Other actions included feed acidification, disinfection of the environment with Virkon S and culling of the most affected pieces not responding to treatment. State of piglet health was improved very quickly.
Another outbreak of the disease took place in November 2015 – after purchase of 10 gilts from a new source farm and repeated worsening of the stable environment, incl. low temperatures and draught. Progression in the weaned pigs was similar to that in spring and nervous symptoms were also reported. *Salmonella takoradi* was again confirmed; this time even in the brain of the dead animals, besides small intestines and rectal swabs. Lawsonia intracellularis and rotavirus of the A group were diagnosed in the weaned pigs.
Results: We unfortunately failed to discover the original source of infection, due to which the stable environment was contaminated. In the latter case co-infection of rotavirus and *L. intracellularis*, most probably dragged by purchase of new gilts from an unaccredited source, participated very likely in outbreak of the disease. Regular application of effective disinfection, leading to reduction of the infection pressure and permanent temperature regime in the piglet stables contributed mostly to improvement of the state of health of the piglets suffering by clinical salmonellosis. In case of deteriorated zoohygiene recurrence can be expected, mainly during spring and autumn months.
Conclusion: Rare serotypes of *Salmonella* including *S. takoradi* can cause disease in swine, but are associated with predisposing factors which allow immunologically naïve pigs to be exposed to very large doses.

Disclosure of Interest: None Declared

Keywords: Salmonella takoradi

Bacteriology and Bacterial Diseases
SALMONELLA

PO-PC01-013

Evaluation of an autogenous Salmonella Typhimurium vaccine in pigs in Northern Ireland

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Introduction: *Salmonella Typhimurium* has been a constant problem on a 1,000 sow unit from birth to bacon. Clinical signs included scour, ill thrift and high mortality during the first 8 weeks post-weaning. Acidification of diets and the use of antibiotics has been the traditional approach for controlling clinical signs of disease. Since the introduction of new genetics in 2014 and PRRSv (Porcine Reproductive and Respiratory virus) in 2015, there has been an increase in clinical signs of salmonellosis and it has been more difficult to control it with the traditional approach. Furthermore, around 10% of pigs in each batch had very poor quality when moving from the weaning to the finishing accommodation.
The aim of the study was to assess the performance of an autogenous *Salmonella* vaccine and compare it to a control of unvaccinated pigs.
Materials and Methods: Faecal samples were taken from all weaner pens and clinically affected pigs with scour. The samples were sent to AniCon Lab GmbH (Germany) for analysis. *Salmonella Typhimurium* was isolated from all the faecal samples. An autogenous vaccine was made for this farm. Two groups were randomly selected. Each group had 500 pigs. In the vaccinated group, the pregnant sows were vaccinated 3 weeks before farrowing. The piglets from these sows were vaccinated a week before weaning. The diet from the vaccinated pigs was not acidified or medicated. The factors taken into account for analytic purposes were the weight in and out, DLWG (Daily Live Weight Gain), FCE (Feed Conversion Efficacy), mortality and number of poor pigs per batch. Data was analysed using JMP version 9.0.3 (SAS Institute Inc., Cary, NC, USA) at a significant level of 0.05.
Results: There was a significant difference ($p<0.05$) between vaccinated pigs (2.3% mortality, 3 poor pigs per batch ie 0.6%) and unvaccinated pigs (5.3% mortality, 42 poor pigs per batch i.e. 8.4%). Results were better in vaccinated pigs when compared with unvaccinated pigs for DLWG (598 vs 519) and FCE (1.37 vs. 1.4).
Conclusion: *Salmonella* is a major cause of food-borne illness in humans. Most salmonellosis outbreaks occur in weaner pigs, and although disease in adults and piglets is infrequent infection is not. *Salmonella* infection in pig herds is much more common than disease and involves limited invasion of tissues, including mesenteric lymph nodes, tonsils, intestine or bladder. This is a very important consideration for both pig and human health and welfare. The results of the present study show that vaccinated pigs thrive more evenly grow faster and there is less mortality. This can indirectly result in the reduced use of antibiotics.

Disclosure of Interest: None Declared

Keywords: Autogenous Vaccine, Salmonella

Poster Abstracts

Bacteriology and Bacterial Diseases
SALMONELLA

PO-PCO1-014

Pig digestive microbiota is different depending on the presence or absence of Salmonella spp in faeces

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Introduction: The microbiota has been extensively studied to decipher its influence in the development of many diseases. *Salmonella* is a main food-borne pathogen causing infections from animals to humans worldwide. Asymptomatic pigs carrying the pathogen in the digestive system are a main source of human infection. The intestinal microbiota of pigs is colonized by an array of bacteria, many of which are poorly described. The role of such microbiota composition in allowing the presence of the pathogen in pig gut is unknown. Hence, the purpose of this work was to characterize the gut microbiota composition of pigs clustered by the presence or absence of *Salmonella spp* in feces under field conditions.
Materials and Methods: Intestinal content samples (IC) were collected at abattoir from 15 pigs of two farms with high prevalence (87%) and from 20 pigs from two farms where *Salmonella spp* was not detected. The pathogen was isolated according to the ISO 6579:2002/ Amd 1:2007, and serotyped in the National Reference Center for Animal Salmonellosis (Madrid, Spain). Microbioma analysis was performed by massive sequencing of V1 and V2 hyper-variable regions of the 16S RNA gene, in a PGM Ion Torrent (Life Technologies) apparatus. Sequencing data was analyzed by QIIME v1.8.0 software, and sequences with ≥97% similarity were clustered in operational taxonomic units (OTUs). Representative sequences were aligned by the PyNAST software, *vs*. the Greengenes 13.8 prealigned database. The results obtained were compared using suitable statistical methods.
Results: *Salmonella* positive IC showed higher (p<0.05) number of bacterial species than pathogen free IC (alfa diversity). Beta diversity analysis showed a significant clustering of positive or negative samples themselves. A total of 21 *phylum* were detected, predominating *Firmicutes* (50.3%), *Bacteroidetes* (38.4%), *Proteobacteria* (5.2%) and *Spirochaetes* (2.4%). *Salmonella* positive samples showed a microbiota increased in *Firmicutes* and *Proteobacteria*, and decreased in *Bacteroidetes*. *Regarding* bacterial families, *Acinetobacter* and *Solibacillus* were the most prevalent in *Salmonella* positive pigs, while *Prevotellaceae* and *Clostridiaceae* were in the uninfected ones. OTUs comparisons showed statistical differences in ten bacterial families, with a significant increasing of *Enterobacteriaceae*, *Verrucomicrobiaceae* and *Pseudomonadaceae* in *Salmonella* positive pigs, and *Mycoplasmataceae* in *Salmonella*-free pigs.
Conclusion: The digestive microbiota composition was significantly different depending on the presence or absence of *Salmonella spp.* This result paves the way to modify microbiota as a tool to control this zoonotic pathogen.

Disclosure of Interest: None Declared

Keywords: Microbiota, Salmonella

Herd Health Management and Economy

PO-PC02-012

Prevalence of respiratory pathogens based on oral fluids on Dutch pig farms with PRDC problems

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Introduction: In the last few years Oral Fluid (OF) testing technology using ropes has been used for the help in diagnosing in PRDC problems. It is a relative cheap, non-invasive method to collect saliva samples in pens with clinical problems, and combined with PCR it can give more insight in pathogens present at time of clinical signs.
The objective of this retrospective study was to get more insight in the prevalence and dynamics of different pathogens in PRDC problems on Dutch pig farms by using rope collection with PCR testing for different pathogens.
Materials and Methods: In the period of November 2013 until October 2015 samples were collected on farms with respiratory problems in pigs. The age of pigs sampled ranged from weaned pigs till slaughter, depending on the onset of the clinical signs in affected pigs. OF samples were taken with ropes, hanging for 20-30 minutes in the pens with the pigs with respiratory problems. Samples were transported overnight by carrier to diagnostic lab (IVD).
In total 108 samples on 47 different sample periods were collected from 27 farms. The samples were analyzed by a multiplex PCR (on PRRS, Influenza, M hyo, PCV2, APP) and reported as negative or positive for the respective pathogen. The results of the samples were divided in 5 age groups: 4-6, 7-9, 10-13, 14-18 and 19-24 weeks of age.
Results: In these farms with clinical signs of respiratory problems different dynamics for the investigated pathogens were observed. For PRRS the peak of prevalence (40% of investigated samples) was in the age groups of 10-13 weeks, closely followed by the age groups 7-9 and 19-24 weeks (both 35%). Influenza was most found at 10-13 weeks (32%), followed by 22% in age groups 7-9 weeks. In Mhyo the 2 highest prevalence's were found in the late finishing groups of 14-18 weeks (65%) and 19-24 weeks (>75%). In the other 3 groups it ranged between 7 and 28%. For PCV2 the peak was found at 7-9 weeks (53%). APP was detected in all age groups, increasing from 57% in the 4-6 weeks of age till 100% of the 19-24 group, except in 14-18 week group were only 17% of the samples were positive.
Conclusion: The advantage of PCR is that you demonstrate in farms with respiratory problems the presence of that pathogen at that time. With that it is not proven it is the leading cause of the clinical signs. Also false negative PCR results can occur. Another aspect is one has to keep in mind non-pathogen factors like climate, housing can cause respiratory problems. Still, this retrospective study gives more insight in the relative importance of different pathogens found in different age groups of pigs on Dutch pig farms.

Disclosure of Interest: None Declared

Keywords: Diagnostic, PRDC, prevalence

Herd Health Management and Economy

PO-PC02-013

Animal level risk factors associated with nursery and finisher mortality

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Introduction: High mortality in the pig industry is a welfare problem and it reduces the farmer's income. The Danish Pig farmers have decided to reduce the mortality with 20 % before 2020 compared to the 2011 level. For each percent the mortality is reduced in the nursery and finisher barns the gross margin per pig is increased by 0.33 euro and 0.70 euro, respectively. The objective of this study was to identify risk factors and estimate their impact on nursery and finisher mortality on population level.
Materials and Methods: The hypothesis was the that duration of the period that a sow entered farrowing unit before farrowing, parity, litter size, gestation period, night and weekend farrowings, back fat of sow, assisted birth, use of oxytocin, MMA and other infections in sows, piglet gender and birth weight, weight at weaning and end of nursery period, cross fostering, moving pigs, and infections in pigs are associated with mortality.
The study was performed as a cohort study in 9 farrow-to-finish herds with more than 1.8 stillborn piglet per litter and ≥ 3% mortality in nurseries or finisher barns before inclusion in the study. Approximately 70 consecutive farrowings in each herd were included in the study. For sows their ID, parity, farrowing data, and treatments were recorded. Each piglet was ear tagged and weighed at birth, weaning and at entry to finisher barn. All pig treatments, movements and deaths were recorded.
The initial statistical univariate analysis was performed by logistic regression on piglet level with herd and sow as random effects. Factors significant at P < 20% were included in the multivariate analysis. To estimate the impact on population level the Population Attributable Risk (PAR) was estimated by a macro that included all the significant risk factors from the multivariate analysis (P < 5%).
Results: A total of 7145 nursery and 5635 finisher pigs were included in the statistical analysis. The mortality in the nurseries and finisher barns were 2.7% and 1.9%, respectively. The estimates of PAR for the nursery showed that without the risk from weaning weight < 5.6 kg the mortality could be reduced from 2.7% to 2.1% (22% reduction). Without the risk from first parity sows, and sows treated for MMA the nursery mortality could be reduced by 12% by each factor. In the finisher barn the mortality could be reduced from 1.9% to 1.5% (19% reduction) without the risk from pigs born in litters < 16 pigs.
Conclusion: This study indicates that the potential for reducing nursery mortality is 40% (2.7% to 1.6%) by focusing on weaning weight, pigs from first parity sows and MMA treated sows. In the finisher barn the potential for reduction was 19% by focusing on pigs from small litters.

Disclosure of Interest: None Declared

Keywords: Pig mortality, Population Attributable Risk (PAR), Risk factors

Poster Abstracts

Herd Health Management and Economy

PO-PC02-014

Pigs at risk: Impact of birth weight increase on survivability and days to market, a simulation model

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Introduction: As a result of high prolificacy in sows piglet birth weight is decreasing by 25 to 35 gram per extra pig in the litter while its variability is increasing. Piglets under a birth weight threshold of 1.13 kg are pigs at risk. They have low survival chances and need more days to market.
The objective of the simulation model was to determine the impact of individual birth weight increase on survivability and days to 100kg.
Materials and Methods: From 3 farms located in Spain, 2331 piglets from 178 litters were followed from birth to slaughter or moment of death. Litter parameters were collected. The pigs were weighed at birth, weaning, end of nursery, end of growing and on the day before the first pigs of the batch went to slaughter. If the pig died, the date, weight and cause were recorded. The pigs were categorized in increasing birth weight classes of 100 g. For each class, frequency, mortality and days to 100 kg was calculated. To each birth weight class 100, 150 or 200 g was added and the mortality and days to 100 kg were recalculated.
Results: The average litter size was 14.3 piglets, from which 13.1 were live born piglets. The average birth weight was 1.46 kg. Eighty three percent of the pigs made it to harvest. By increasing the birth weight class by 100, 150 and 200 g, the survival rate would increase to 85.6, 86.6 and 87.6% respectively, or 0.34, 0.47 or 0.60 piglets per litter. The litter weight at birth would increase from 19.2 to 20.5, 21.1 and 21.8 kg respectively. Time to reach 100 kg would decrease from 178.7 days to 176.1, 174.5 and 172.9 days respectively. Pre-weaning survival chances of pigs at risk (<1.13 kg) are low (58%) compared to the other piglets (92%). By increasing the birth weight of the piglets proportionally, survivability increases more pronounced in the low birth weight range and this could potentially bring the average growth rate down. However, in the model, the time to market still decreased marginally. As a result, more pigs would reach the market without a negative impact on time to market. By only increasing the birth weight of the pigs at risk with 100, 150 and 200 g, the impact is still 88.5%, 83.3% and 80.4% of the total survival increase and without a change in the days to market (178 days).
Conclusion: The model used suggests that increasing birth weight proportionally has a positive impact on survivability without having a negative impact on the number of full value pigs. Technologies that help to increase the birth weight of pigs at risk only would still have a major impact on overall survivability.

Disclosure of Interest: None Declared

Keywords: Birth weight, Days to market, Survivability

Herd Health Management and Economy

PO-PC02-015

Temporal evolution of production parameters and pig production cost form 2010 -2014 in Spain.

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Introduction: The pork industry is facing lower profit margins per pig, or negative profits with prices lower than marginal production costs, from time to time. Any decision in pig farming should be based on an assessment of the cost of production and the relative weight of the different production parameters to decrease this cost as much as possible. The goal of the present work was to describe the temporal evolution of production parameters and pig production cost from 2010 to 2014 in Spain.
Materials and Methods: Between 61 and 107 pig production companies from Spain were included in this study from 2010 to 2014. These companies sent data on feed consumption, number of pig produced, expenses and census every month. Sip consultors SL standardized collected data and calculate cost and production parameters to obtain values comparables between the different pig production companies. The collected data each month were merged to obtain a yearly average value taking into account the pig production flow each month. A statistics descriptive was calculated for each parameter during the period 2010 to 2014. An Anova or Wilcoxon test was used to analyse the association between continuous normally or non-normally distributed variables and year. Finally, a linear model was performed to evaluate the association between the production parameters and the year taking into account that the data was recorded each year (repeated measures) and the potential interaction between year and pig company.

Results: The production performance has been continuously improving in the piglet production and fattening phase from 2010 to 2014. Thus, the number of piglets by sow and year will increase 0.5 pigs by year and the global feed conversion rate will decrease approximately 0.03 by year in the future if the same tendency continues. However, feed price has been steadily increasing from 2010 to 2012 and decreasing afterwards and the total cost per kilogram produced has followed a similar pattern. Using linear model analysis, number of piglets born alive, number of piglets weaned per sow and number of piglets produced by sow and year were positively associated with the year (the older the year is, the higher the value is). On the other hand, fattening feed conversion rate, global feed conversion rate, fattening mortality and kilograms of sow feed per weaned piglet were negatively associated with the year (the older the year is, the lower the value is).
Conclusion: Pig production parameters have generally improved in the last five years. During the period 2010-2014, this improvement did not directly imply a reduction in pig production cost due to the high feed prices.

Disclosure of Interest: None Declared

Keywords: Evolution, production parameters, Year

Herd Health Management and Economy

PO-PC03-007

Clinical aspects and weight gain reduction in swine infected with porcine circovirus type 2 and torque teno sus virus in Brazil

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Introduction: The impairment of weaning and fattening parameters in commercial pig farms results in economic losses, higher food intake and increased feed conversion ratio. Among viral infections responsible for weight loss, Porcine circovirus type 2 plays an important role as a virus responsible for systemic disease or subclinical infection. Simultaneous Porcine circovirus type 2 (PCV2) and Torque teno sus virus (TTSuV) infections have been reported with increasingly frequency around the world, generally linked to more severe infections. Therefore, this study aimed the investigation of PCV2 and TTSuV 1 and 2 presences in serum samples of domestic pigs, and its correlation with clinical signs and weight measurements.
Materials and Methods: We conducted a transversal study involving 257 animals from 31 swine herds located in Rio de Janeiro State, Brazil. PCV2 vaccine was not included in the vaccination protocol of the herds. Clinical examination and weighing were performed before blood collection and all data were recorded for further analysis. A total of 244 animals were weighed and the measured weight was compared to the average weight of the pen. During sample collection, 150 animals showed clinical signs (enteric; respiratory; and multiple signs, defined as a combination of both), while 107 did not show any clinical symptoms.
Results: PCV2 was detected in 25% (being 73.3% PCV-2a and 26.7% PCV-2b), followed by 38.1% and 42.4% of TTSuV1 and TTSuV2, respectively. Co-infections of two or three viruses were found in 32.3% of samples. PCV2 was more frequently detected in the growing ($p=0.030$) and finishing phases ($p=0.0005$) while TTSuV2 in the nursery ($p=0.009$). Only TTSuV1 was statistically associated to clinical disease (multiple signs), in combination or not with PCV2 or TTSuV2 ($p=0.015$). PCV2/TTSuV co-infections were more frequently related to weight gain reduction in comparison to mono-infections ($p=0.049$) and no-infections ($p=0.027$), and also in animals with ($p=.011$) or without ($p=0.037$) clinical signs, being the nursery the most affected phase ($p=0.025$).
Conclusion: This is a largest study to investigate the clinical effects of PCV2/TTSuV infections in Brazil. Our results confirms the circulation of PCV2, TTSuV 1/2 genotypes in Rio de Janeiro, highlighting the negative impact of these viral agents in pig farms, especially in early production phases. The clinical manifestations associated to these agents and the reduced weight gain in both symptomatic and asymptomatic co-infected animals, advocate for the implementation of large scale control measures in order to prevent economical losses.

Disclosure of Interest: None Declared

Keywords: PCV2, TTSuV1/2, weight gain

Herd Health Management and Economy

PO-PC03-008

Practical ways to produce key information to enable process control in a pig finishing system

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Introduction: Profitability of finishing pig production is determined by relatively small differences in physical performance of Key Profit Determining factors together with market prices of inputs and output. Many producers – at best have batch performance data – this is derived too late for positive interventions to be made to influence performance. What is required is derivation of meaningful data during the production of a batch of pigs enabling corrective appropriate action to be taken.
Materials and Methods: An 1800-finisher pig place straw-bedded, naturally ventilated (ACNV) house managed on an all in-all out basis comprising 20 pens housing either 90 gilts or boars on a split-sex basis. Pigs entered at approximately 53kg and were sold at 112kg live weight. Pigs were fed ad libitum, 2 sample pens were weight monitored at weaning, on entry to finishing and weekly for the last 8 weeks of the finishing period. One such pen had an in-pen auto weigher (Schippers Ltd) enabling sample weighings on a daily basis to be made. Two bulk feed bins supplying feed to 20 pens were also monitored via load cells; water usage was monitored daily using electric pulse meters. This study examined the practicalities of deriving process control data in a commercial pig finishing facility.
Results: Pigs entered the 2 test pens at a mean weight of 56.2kg SD 8.6kg (31.5-86.5kg) and 15% coefficient of variation. Pigs were sold over a period of 44 days and 7 sales draws / pen to optimise mean sale weight at 110.5kg. Auto-weigher: pig usage rate varied between batches, daily mean visits ranged between 32 and 121 pig visits / day / batch. Higher visit rate improved accuracy of weighings which ranged from 97-106% of actual pig weighings, compared to 92-106% of actual weight for lower frequency visits. With high usage rates approximately 75% visited the weigher daily. Bulk feed bin weigher use enabled daily feed intake (DFI) to be monitored, use of a rolling 7 day average daily intake proved to be less erratic than daily intake data. A mean daily feed intake of 3.1kg day at a mean live weight of 85kg resulted in a DFI of 3.6% of body weight. Water intake monitoring: daily intake was more variable than anticipated; again a rolling 7 day mean is advised. Water intake was 2.4 times that of feed intake and 8.6% of body weight.
Conclusion: The equipment used to measure input and performance was reliable though daily data needs to be interpreted with caution, rolling 7 day averages are preferable. Once several batches are completed a comparative benchmark can be utilised to highlight deviations in daily performance.

Disclosure of Interest: J. Richardson Conflict with: Consultant

Keywords: Automation, Monitoring, Performance

Poster Abstracts

Herd Health Management and Economy

PO-PC03-013

Corrosive action of iron bisglycinate-chelate on the gastro-intestinal mucosa in piglets

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Introduction: Newborn piglets have small iron reserves, get low iron amounts by sow milk but grow rapidly and live indoors. Thus, without extra iron supplementation piglets will become anemic within few days. Although intramuscular application of iron dextran is the most common supplementation method, oral iron application avoids injections with possible entry of infectious agents, and is in compliance with ecological animal husbandry. The corrosive effect of iron sulfate, the most used salt for oral iron supplementation in humans is well known. It can cause gastrointestinal mucosal erosions also in normal dosage. Therefore, less aggressive iron compounds are propagated. A newly designed Swiss oral iron paste on the basis of iron bisglycinate chelate (FeBiGly) was used in newborn piglets. The European Food Safety Authority (EFSA) considers the use of ferrous bisglycinate, meeting proposed specifications (77% FeBiGly, 17% citric acid, daily intake of 4-6 mg Fe/day for children and 8-10 mg Fe/day for adults) as safe. However, in some farms many piglets died after the oral application of the paste.
Materials and Methods: The paste contained 180 mg FeBiGly per portion (approx. 36 mg iron/kg; approx. 6.5% of the LD50 in rats) and was orally administered at day 1-3 p.p. Twelve dead piglets were necropsied, all with macroscopically evident gastric lesions. To prove that FeBiGly was the causative agent, an animal experiment was carried out with one litter of 11 piglets divided in 2 controls and 6 piglets receiving 180 mg FeBiGly and 3 piglets receiving 360 mg FeBiGly 24h p.p. Euthanasia was performed at 24h, 48h and 72h after iron application always for 2 piglets with 180 mg FeBiGly and one with 360 mg FeBiGly. The two controls were euthanized at day 2 and day 3, respectively.
Results: The necropsied piglets from the field showed macroscopically impressive lesions in the stomach. All piglets with experimental iron paste application showed also macroscopically visible lesions in the gastric mucous membrane. However, the lesions induced experimentally were with one exception macroscopically less severe. Histologically, all piglets that were fed with the iron paste showed necrotizing gastritis but of different severity.
Conclusion: Iron sulfate is a common cause of fatal poisoning in children by accidental ingestion of tablets and necrotizing gastric and intestinal mucosa damage is illustrated by histology in published case reports. Many of our cases showed similar histologic alterations with the same morphological severity as the described cases in children with iron sulfate intoxication. However, in the piglets the iron compound was the designated "safe" ferrous bisglycinate chelate.

Disclosure of Interest: None Declared

Keywords: oral iron application, toxicity

Herd Health Management and Economy

PO-PC03-014

Sow mortality and meat inspection findings of Finnish sows

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Introduction: This study aimed to describe meat inspection (MI) findings of Finnish sows and to examine the possible association between sow on-farm mortality and MI findings.
Materials and Methods: Mortality and MI data was collected for the year 2014 from 39 farms. Herd-level numbers were obtained from the national swine register, where farmers report their monthly numbers of females (sows and gilts) alive, dead/euthanized and slaughtered. A 12-month mean value of the number of females was calculated. The summary for the 12-month period was calculated for dead, euthanized or slaughtered animals. Mortality was defined as the number of dead and euthanized animals divided by the mean number of females in the herd. Low (LM) and high mortality (HM) herds had a mortality of less or more than median of the mortality of all herds, respectively.
Three slaughterhouses provided their records: numbers of animals slaughtered, carcass weight, lean meat percentage, MI findings, condemnations and kg of meat condemned. Multivariable models were built for outcomes.
Results: The herds had an average of 529 (sd 479) females per farm. A total of 2 211 animals died or were euthanized during the study period yielding an average annual on-farm mortality of 9.0% (sd 5.2). The herds sent 7 531 animals to slaughter and we were able to gather MI records from 98.8% of them.

The mean carcass weight was 188.8 (sd 12.9) kg and median lean meat % 60.0 (48.8-63.4, min-max). Altogether 22.8% (0-49.3%) of the slaughtered animals had at least one MI finding .When mortality increased by 1%, the percentage of females with at least one MI finding increased by 0.9% (p=0.01). The whole and partial carcass condemnation percentages were 1.8% (sd 1.9) and 11.8% (sd 7.4), respectively. When herd level mortality increased by 1%, the percentage of partial condemnations increased by 0.4% (p=0.08). Altogether 38 038 kg of meat (975 kg per herd) was condemned due to some reason. MI revealed following findings (median, min-max): arthritis 2.1% (0-13.3), abscess 5.7% (0-16.3), pneumonia 1.0% (0-3.6), pleuritis 1.7% (0-36.4), liver 0% (0-11.3), organ 0% (0-13.3) and skin ulcer 3.6% (0-22.9). Even though HM herds had more meat inspection findings, the only significant association between the findings and herd mortality was found for pleuritis: HM herds had a greater risk for having higher percentage of pleuritis (OR 3.9, p=0.05) than LM herds.
Conclusion: A considerable percentage of females either died or were euthanized in the herds. Furthermore, a large amount of sow meat was condemned. Large variation between herds existed in the results. HM herds were more likely to have higher pleuritis figures than LM herds. MI data could be used more in herd health work.

Disclosure of Interest: None Declared

Keywords: meat inspection, mortality

Herd Health Management and Economy

PO-PC03-018

Evaluating disease behavior and health interventions using pattern classification of in-process performance in animal cohorts for flows and systems.

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Introduction: Producers typically evaluate records to determine if farm, flow and system performance is meeting expectations and to detect problems. For both reproductive sites and growing sites, these evaluations typically involve assessing time-series reports as well as cohort-based reports (e.g., breeding and growing animal groups). Time-series reports can be useful, but are inadequate for enabling timely and targeted responses to detected problems. Cohort-based reports are better suited for timely detection of in-process problems, but lack objective rigor, appropriate orientation or both. A model was developed to categorize cohort performance patterns and detect in-phase shifts of tracked measures (e.g., mortality, morbidity, clinical events, feed/water consumption).
Materials and Methods: To construct the algorithm for pattern characterization, cohort start date is treated as time zero, and interval segments are user-defined. For each interval segment, a time series chart is generated and statistical process control (SPC) calculations are applied to each set of data, resulting in a time-series SPC chart for each interval. Then the control limits from each time-series interval SPC chart are aggregated into a composite chart, representing the entire cohort period. From this aggregate SPC chart, all control limits are standardized (0,1) and standardized values are then calculated for all individual data points. Using a rule-based algorithm, all standardized data points are classified as Low (L), Middle (M) and High (H). A three-interval pattern classification matrix was developed for all combinations of LMH, resulting in a total of 27 patterns. These 27 patterns were then ordinally scaled from least (LLL) to most (HHH) serious, and were further consolidated into eight distinct categories. A pilot project was conducted utilizing daily mortality data obtained from two producers. Cohorts were classified by type of mortality pattern and category within specific production phases.
Results: This cohort methodology and model was used to dynamically generate pattern classifications for cohort-based measurments. It was then used to conduct mortality pattern analysis within and among source data producers/production systems.
Conclusion: To further evaluate and leverage the methodology, the cohort model will combine leading, early clinical, lagging clinical and ending cohort indicators (e.g., water consumption, cough, mortality, treatments, closeout performance) with systematic animal and environmental sampling/diagnostic testing results; the objective being to improve operational decision-making and intervention design.

Disclosure of Interest: None Declared

Keywords: None

Herd Health Management and Economy

PO-PT2-010

Animal level risk factors associated with preweaning mortality

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Introduction: High mortality in the pig industry is a welfare problem and it reduces the farmer's income. The Danish Pig farmers have decided to reduce the mortality with 20% before 2020 compared to the 2011 level. For each percent the mortality is reduced in the farrowing units the gross margin per sow per year is increased by 6 euro. The objective of this study was to identify risk factors and estimate their impact on preweaning mortality on population level.
Materials and Methods: The hypothesis was that the duration of the period that a sow entered farrowing unit before farrowing, parity, litter size, gestation period, night and weekend farrowings, back fat of sow, assisted birth, use of oxytocin, MMA and other infections in sows, piglet gender and birth weight, and infections in pigs are associated with preweaning mortality.
The study was performed as a cohort study in 9 farrow-to-finish herds with more than 1.8 stillborn piglet per litter. Approximately 70 consecutive farrowings in each herd were included in the study. For sows their ID, parity, farrowing data, and treatments were recorded. Each piglet was weighed and ear tagged at birth. All pig treatments and deaths were recorded.
The initial statistical univariate analysis was performed by logistic regression on piglet level with herd and sow as random effects (Proc Glimmix in SAS). Factors significant at P < 20% were included in the multivariate analysis. To estimate the impact on population level the Population Attributable Risk (PAR) was estimated by a macro that included all the significant risk factors from the multivariate analysis (P < 5%).
Results: A total of 8810 live born piglets were included in the statistical analysis and 1615 piglets died in the farrowing units (18.3%). Gender was forced into the multivariate analysis. The multivariate analysis showed that assisted farrowings, gender, low birth weight and parity was associated with preweaning mortality at P< 5%. The estimates of PAR showed that without the risk from assisted farrowing the preweaning mortality could be reduced from 18.3% to 17.4% (5% reduction). Without the risk from male pigs, sows > third parity, and small pigs < 1 kg birth weight the preweaning mortality could be reduced by 6%, 15%, and 50%, respectively.
Conclusion: This study indicates that the potential for reducing preweaning mortality is 62% (18.3 to 7.0%) by focusing on farrowing process, low weight piglets, older sows, and male pigs (castration).

Disclosure of Interest: None Declared

Keywords: Population Attributable Risk (PAR), Preweaning mortality, Risk factors

Poster Abstracts

Herd Health Management and Economy

PO-PT2-027

Pigs at risk: Impact of birth weight on weight gain until harvest

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Introduction: A strong focus on litter size selection and management has resulted in a steady increase of the number of piglets weaned per sow per year. High prolificacy in sows and increased fetal survival however leads to intra uterine crowding and growth retardation. As a result the piglet birth weight (BW) is decreasing and its variability increasing. For every extra pig in the litter, the average piglet BW drops with 25 to 35 gram. Low BW has been defined as piglets beneath a certain weight cut off but there is no uniformity throughout the published studies.
The objective of this study was to investigate the relation between the individual BW of the piglet and its weight gain up to slaughter.
Materials and Methods: From 3 farms located in Spain 1338 piglets from 178 litters completed the trajectory from birth to slaughter. Litter parameters were collected. The pigs were weighed at birth, weaning (26 days), end of nursery period (67 days), end of growing period (110 days) and the day before the first pigs of the batch went to slaughter (157 days). Days to 100 kg life weight were calculated based on the life daily gain. Descriptive statistics were applied to evaluate the impact of BW on weight gain.
Results: Average BW of all pigs was 1.46 kg. Farm and litter size had an impact on BW. The total mortality was 17.5% and these are not included in the weight analysis. The survival chances under the breaking point of 1.13 kg were very low (58%). Above that threshold the survival rate was 92%. As a result, the average BW of the pigs that finished the trajectory was higher (1.54 kg). The surviving piglets up to 1.13 kg at birth (10 % of total) had an average daily gain of 203, 293, 601 and 805 g for the lactation, nursery, growing and finishing period respectively and the time to reach 100 kg was 191 days. Piglets of 1.13 kg and higher (90 % of total) had an average daily gain of 228, 359, 659, 845 g for the lactation, nursery, growing and finishing period respectively and the time to reach 100 kg was 177 days. The daily gain of the low weight piglets was constantly lower during each growing phase, showing no compensatory growth potential.
Conclusion: Apart from low survival chances, pigs at risk (BW pigs < 1.13 kg) have a negative impact on the days to market and on the uniformity of the batches. This leads amongst others to a decrease of efficient use of the farm facilities.

Disclosure of Interest: None Declared

Keywords: Birth weight, Weight gain

Herd Health Management and Economy

PO-PT2-037

Assessing economic benefit of preventive PRRSv immunization strategies

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSv) causes significant economic losses in pig production in most pig producing countries with a few exceptions.
Aiming to reduce PRRSv impact, veterinarians developed strategies to control or eliminate infection from pig populations. In the North American swine industry it is a common practice the adoption of “herd closure” (interruption of pig introduction into the herd) associated with “whole herd exposure” to a modified-live virus (MLV) vaccine or live field-virus inoculation (FVI), method commonly referred to as “load-close-expose”.
It has been reported that herds that used MLV as part of load-close-expose program produced PRRSv-negative pigs at weaning 7 weeks later, but recovered productivity levels 11 weeks before those that used FVI. It was also reported that herds that had prior immunity to PRRSv recovered significantly faster and produced PRRSv-negative before herds without prior history of PRRSv infection.
Materials and Methods: We developed economic models using partial budget and Monte Carlo simulation methods to compare MLV to FVI as the exposure method of load-close-expose program to control and eliminate PRRSv from infected breeding herds.
We also built economic models to estimate benefit over cost of the strategy to vaccinate sow herds preventatively (with intent to build anti-PRRSv prior immunity).
The models were based on field data collected from our previous studies and/or from literature.
Results: *MLV vs FVI.* Under the assumptions plugged in the models, MLV held economic advantage over FVI on 60% of the time. Nevertheless, sensitivity analysis revealed that decreasing margin over variable costs below $ 47.32, or increasing PRRSv-attributed cost above $18.89 or achieving time-to-stability before 25 weeks resulted in advantage of FVI over MLV.
Economic value of preventive vaccination. Preventive vaccination of sow herds was beneficial when the frequency of PRRSv infection was at least every one year and 9 months. Economics of preventive vaccination was minimally affected by cost attributed to field-type PRRSv infection on growing pigs or by the breeding herd productivity level.
Conclusion: Results showed overall advantage of MLV over FVI as part of load-close-expose programs. However, results were sensitive to changes in some key parameters. Preventative vaccination was economically viable when frequency of PRRSv infections was less than 1.75 years. Altogether, results allow veterinarians and producers making informed decisions in their efforts to control or eliminate PRRSv infections from production systems.

Disclosure of Interest: None Declared

Keywords: herd immunity, PRRS, Vaccination

Herd Health Management and Economy

PO-PT2-038

Comparative efficacy of a ready to use and freshly mixed PCV2 and Mycoplasma hyopneumoniae vaccine

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Introduction: A ready to use vaccine combining PCV2 and Mycoplasma hyopneumoniae (Mhyo) has recently become available. The objective of this study was to compare the efficacy of this vaccine against a PCV2 and Mhyo vaccine licenced to be mixed on farm under commercial farm conditions in a UK pig farm.
Materials and Methods: An indoor farrow-to-finish unit was selected for this study following confirmation that PCV2 and Mhyo were circulating on the farm. A total of 672 pigs were assigned to one of three groups, group P (Porcilis® PCV M Hyo, 2mL IM, n=225), F group (PCV2 and Mhyo licensed to be mixed on farm, 2 mL IM, n=226) and control group, group C (sterile saline, 2mL IM, n= 221). Pigs were weighed and blood samples were collected at inclusion, nursery and before slaughter. The lungs of the pigs were examined and scored for typical Mhyo lesions (EPL) according to the method of Goodwin and Whittlestone (1967). Data was analysed using proprietary statistical software (SAS).
Results: Live weight was not significantly different between the three groups at inclusion or nursery stage. At slaughter, P pigs (86.4 kg) were heavier than F (83.6 kg) and C (84.4 kg) pigs. Average daily live weight gain (g/day) (ADWG) from inclusion to slaughter (O) and from nursery to slaughter (FIN) was significantly higher for P pigs than for F (p = 0.0006, 0.0055) and C (p = 0.0006, 0.0055) pigs (O - 576, 558, 563 g/day and FIN - 682, 656 and 661 g/day respectively). The EPL were significantly lower for P (2.95) than F (5.44; p=0.0002) and C (4.92; p=0.0009) pigs. Live weight pre-slaughter, ADWG and EPL were not significantly different between C and F pigs (p>0.05).
Conclusion: The difference in performance between P and, F and C pigs is likely to be due to a better Mhyo control, as supported by Mhyo seroconversion and lower EPL in P pigs. The EPL level following vaccination with Porcilis PCV M Hyo was just below the score during the pre-trial assessment (a reduction of 1/55), when pigs were vaccinated with a different oil based Mhyo vaccine. Absence of PCV2 seroconversion in the control pigs indicates a very low field challenge. The study demonstrated that Porcilis PCV M Hyo was effective and convenient to control Mhyo and PCV2 in a commercial UK pig farm.

Disclosure of Interest: R. Neto Conflict with: emplyee, R. jolie Conflict with: employee, E. Van Hee: None Declared, R. Evans: None Declared, D. Berkshire: None Declared, N. Woolfenden: None Declared

Keywords: hyopneumoniae, Lesions, PCV2

Herd Health Management and Economy

PO-PT2-039

Long-term effects of colostrum intake in piglet mortality and performance

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Introduction: Colostrum intake (CI) by neonatal piglets is essential as colostrum is the sole external energy supply and provides immunological protection and growth factors. It is well known that insufficient CI is a major cause of pre-weaning mortality. Some authors have presumed long-term effects of CI on mortality and performance. However, studies investigating such long-term effects of CI are still scarce. Furthermore, most trials on colostrum in pigs are conducted under experimental conditions or in one commercial herd. Therefore, this study aimed to investigate the short-term as well as the long-term influence of CI on performance and mortality on 10 commercial Belgian pig herds.
Materials and Methods: A total of 1,455 live born piglets were followed-up from birth until 22 wk of age. Pigs were individually weighed at birth, at weaning, at onset (intermediate weight) and during the fattening period (finishing weight). One linear mixed model was fitted to model the possible associations between CI and weight at weaning, intermediate and finishing period. In addition to CI as main predictor of interest, also other predictor variables were tested namely birth weight, birth order, sex, breed and the interval between birth and first suckling **(t_{FS}).** Three generalized linear mixed models were performed to model the probability of dying either during the suckling, the nursery or the fattening period. Colostrum intake, birth weight, birth order, sex, breed and t_{FS} were tested.
Results: Colostrum intake and birth weight were positively associated with weaning (P<0.001), intermediate (P<0.001) and finishing (P<0.001) weight. Furthermore, higher CI is more beneficial to weaning (P <0.001), intermediate (P <0.001) and finishing (P =0.02) weight in piglets with low versus high birth weights. Birth order was positively associated with weight at each time (P=0.01). Sex was only significantly associated with finishing weight (P<0.001). Some breeds differed in weight at onset or during the fattening period. The association between t_{FS} and weaning weight differed by breed. Pre-weaning mortality was negatively associated with CI (P<0.001) and birth weight (P=0.004) and positively with t_{FS} (P<0.001). Mortality during the nursery period was negatively associated with CI (P<0.001) and birth weight (P=0.002). The negative association between CI and mortality during the suckling (P<0.001) and nursery (P=0.008) period was more pronounced in small versus heavy piglets.
Conclusion: In conclusion, CI significantly influences performance and mortality in the short-term as well as in the long-term. As colostrum yield is reported to be independent of litter size, sufficient CI per piglet is crucial especially in hyper-prolific sows.

Disclosure of Interest: None Declared

Keywords: Colostrum, long-term, piglet performance

Poster Abstracts

Herd Health Management and Economy

PO-PT2-045

Medication via drinking water by a dosing pump : motivations, obstacles and use practices

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Introduction: While antimicrobial treatments administered via the feed were largely predominant in France 15 years ago, drinking water is becoming the main administration route, as 51% of treatments are found as oral solutions and powders in 2013 against 22% in 1999. Mostly administered by dosing pump, the reliability of these treatments requires adapted material and correct use practices. This study has for objective to describe the motivation and use practices of the dosing pump in a sample of equipped farms in 2014. It also aims to understand the obstacles for non-equipped farms.
Materials and Methods: A phone survey was conducted in a sample of 109 French farms equipped with a dosing pump and 46 farms which were non-equipped.
Results: One of the main reasons cited by 46% of farmers for acquiring a dosing pump is the higher efficiency and the shorter implementation of treatment administered through water over feed. Regarding the use practices, the best applied recommendations are the preparation of the tank solution for a use up to 24 hours (91% of farms) and the complete water rinsing of the circuit at the end of the treatment (90% of farms). In 26% of farms, calculation of the amounts of water drunk by pigs under treatment is relied on the animals' real level of water consumption based on the reading of the water meter or the graduations on the tank. Other farmers (40%) give an estimate of water drunk from theoretical values (10% of the live weight of animals to treat), which is more approximate because based on a constant set for healthy pigs. And 30% of farmers did not explain how they do the calculation for they work "from habit".
Other recommendations are seldom followed by the surveyed farmers whereas they can prevent treatment under-dosing and clogging of troughs and pipes. Indeed, throughout the past year, the trough flow was never controlled in 53% of farms. Occasional solubilisation problems of the treatment, which occurred in 41% of farms, can be explained by some risky practices: the absence of a mixing tank in 31% of farms, no solubility test for new drugs with water from the farm in 59% of cases, mixing of two drugs in 18 % of farms. Finally, improvements are expected in equipment maintenance which is implemented in less than 25% of farms.
For 43% of non-equipped farmers, the main obstacle is the constraint related to equipment: cost, set up, use. Only 11% of non-equipped farmers plan to purchase a dosing pump in the short-term.
Conclusion: This survey helps to better target communication routes to optimise the reliability of treatments administered by a dosing pump, as well as to assist the non-equipped farmers through the acquiring process.

Disclosure of Interest: None Declared

Keywords: Dosing pump, Motivations, Use practices

Herd Health Management and Economy

PO-PT2-046

SUISsano: A pilot project to evaluate and control antibiotic consumption on pig farms in Switzerland

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Introduction: Due to raising antimicrobial resistance there is growing concern about antibiotic use in pig production. A monitoring system was developed in order to evaluate and control antibiotic consumption in Swiss pig production.

Materials and Methods: 20 pig producing farms voluntarily took part in this project. Antibiotic consumption was reported separately for four age groups (piglets, weaned pigs, fattening pigs and sows) by delivery receipts of the veterinarian. Farmers had to report size of livestock and number of produced animals per year.
European Medicines Agency (EMA) has not yet completely assigned technical units for antibiotic consumption (DCD_{vet}). Therefore based on Specific Product Characteristics (SPC) preliminary Defined Course Doses for Switzerland (DCD_{CH}) were described for all drugs containing antibiotic components. Standard weights were defined (Sows: 220kg; piglets: 2kg; weaned pigs: 10kg; finishing pigs 25kg).
The number of DCD_{CH} in proportion to size of livestock (sows) or number of produced animals (piglets, weaned pigs, finishing pigs) was calculated for each farm and age group per year. This treatment index was converted into a points based system in order to provide a most simple and clear unit to the farmers: 1DCD_{CH}/pig/year was defined to be 1000 Points.
The index was reported to the farmers for all age groups separately. With the aim of encouraging farmers to reduce treatments using High Priority Critically Important Antimicrobials (HPCIA), point values of such treatments were multiplied with factor four.
Trends of antibiotic consumption were measured by the number of DCD_{CH} per pig per year in 2013 an 2014.
Results: Overall in this study antibiotic consumption was reduced by 23% in 2014 compared to 2013. The use of HPCIA was reduced by 33% in suckling piglets and by 32% in all age groups.
Conclusion: Analysis using the SUISsano points based system provided the farmer with a simple and useful assessment concerning antibiotic consumption compared to other farms. The marked reduction in the use of HPCIA demonstrates that valuing specific agents by multiplying corresponding treatments with different factors was an effective control tool. Factors can be adjusted according to current or future requirements.
Showing farmers their individual status concerning antibiotic use gives valuable impulses for veterinary advisory and helps implementing Good Agricultural Practice.
The collected data is also appropriate to be converted into the technical units DCD_{vet} of the EMA, which are not yet defined, allowing future comparison to other EU countries. In 2016 the SUISsano System will be launched nationwide in Switzerland.

Disclosure of Interest: None Declared

Keywords: antibiotic reduction, monitoring, Suissano

Herd Health Management and Economy

PO-PT2-047

Prevalence of lameness and type of lesions in sows in Spanish farms

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Introduction: Since the implementation of EU Animal Welfare Legislation an increase of culling of sows because of lameness has been steadily detected. Moreover, almost 40% of non-clearly defined reproductive problems as cause of culling might be highly associated with lameness. For these reasons, the objective of this abstract is to provide a description of the current incidence of lameness and the kind of lesions in sows.
Materials and Methods: For this study, a total of 8 Spanish farms were selected and 10% of reproductive sows in each farm evaluated (187 sows in total). Lameness assessment was conducted following a specific scoring system used by Zinpro® Corporation. Different types of lesions were assessed including severity (1 = low, 2 = moderate, 3 = severe). Lesions assessed were toes length (T); length and lesion in dew claw (DC); Heel overgrowth and erosion (HOE); Heel-sole crack or separation at the juncture (HSC); separation at the white line (WL); horizontal (CWH) or vertical (CWV) crack in the toe wall. Total percentage of each lesion and percentage by parity were calculated and compared by chi-square test.
Results: On the one hand, the study shows a 91.98 % of scored sows affected by some kind of claw lesion. Also, the type of lesion is unequally distributed: the most common injury is HOE with 71.66%, followed by DC=62.6%; CWH=38.5%; WL=26.2%; CWV=23.5%; T= 20.3% and HSC=17.1%. On the other hand, there are significant differences in lesion prevalence related to parity. The most affected sows in each farm are from parity 2 (97.2%; *p=0.011*), followed by parity 6 or more (92.1%) and parity 1 (85.7%) of injured sows. Gilts are the group with the lowest incidence (62.5%), close to parity 3-5 group (64.8%).
Conclusion: The percentage of sows with some kind of claw lesion is higher than it was expected. Also, the parity lesions distribution showed that the incidence of lameness and claw injury are extremely high in parity 2 sows. This could be related to the second parity syndrome, but this needs to be confirmed by further research
Finally, and in order to promote easier knowledge extension and sharing, all data are uploaded and automatically processed and analyzed into a website where veterinarians can check farm's results and benchmark to others for free (www.lamenesscontrol.com).

Disclosure of Interest: None Declared

Keywords: Culling, Lameness, Sows

Herd Health Management and Economy

PO-PT2-050

A model for determining optimal sampling protocols targeting detection of new disease introduction into expected negative animal populations.

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Introduction: For animal production sites (especially those of very high value, such as genetic production and breeding/reproduction sites), that are expected to be and remain negative for a particular disease agent, an appropriate detection plan for new introduction of undesired disease agents must include both continuous clinical observation and well designed diagnostic sampling/testing protocols. Whereas basic sample size determination methods for disease detection from single samplings are generally understood, the factors that contribute to appropriatly sized and timed sampling are less well understood and frequently poorly applied in the design and execution of protocols. A stochastic model was developed to improve sampling protocol development targeting detection of new disease introduction into expected negative animal populations.
Materials and Methods: An algorithm described by Rothman and Greenland (1998) was modified and incorporated into a tool built to stochastically model onset of detection of new disease agent introduction in expected negative animal populations. An animal isolation scenario was modeled where a new cohort of 500 replacement females are moved into an empty site every 60 days, where no live animals exit to a downstream site during the first 30 days and animals are moved from the isolation to the downstream site as needed over the second 30 days of the overall 60 day period, after which the isolation site is empty and sanitized in preparation for the next incoming group of replacement females. Model scenarios were compared by varying index positives at entry (1 or 5 animals), contact probability (30% or 70%), transfer probability (30% or 70%), detection onset lag (2 or 3 days) and detection duration (14 or 21 days). Detection probability curves were generated across a 60 day period for each scenario at sample sizes of 15, 30, 45 and 60. For the sample sizes evaluated, the cohort day at which $\geq$95% of model runs were detected as positive was used as the criteria for comparing scenarios.
Results: Two scenarios comparing 5 or 1 index positives (both with 30%/70% contact/transfer probabilities and 2/21 day detection onset/duration) at a sample size of 30, the 95% detection threshold was achieved at the 15th and 23rd cohort day, respectively.
Conclusion: This stochastic sampling protocol model can be used to derive more informed and appropriate detection sampling protocols for the detection of new disease agent introduction into expected negative animal populations, as well as generate tables to be used as references for disease detection sampling that take into account the dynamics of exposure and transmission in animal cohorts.

Disclosure of Interest: None Declared

Keywords: None

Poster Abstracts

Herd Health Management and Economy

PO-PT2-054

Tail biting is related to respiratory disease at a herd level but not at an individual level

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Introduction: The primary function of meat inspection is the protection of public health. However, there are little data available with primary relevance to animal health or welfare. Carcass tail lesions have potential as 'iceberg' indicators of pig health and welfare on farm. The aim of this study was to assess the relationships between tail lesions and viscera condemnations in an Irish abattoir.
Materials and Methods: The following data were collected at the evisceration point from every 3rd pig slaughtered over 7 days: farm identification, sex, tail lesion score and viscera inspection outcome. Tail lesions were scored according to a 0 to 4 point scale. Disease lesions responsible for lung (pleurisy, pneumonia and abscess), heart (pericarditis) and liver (ascariasis) condemnation were recorded based on the decision of the Veterinary inspector (VI). Data on 3,143 pigs from 61 batches were available. The relationship between disease and tail lesions was studied at individual carcass and batch level.
Results: Tail lesions (score ≥ 1) were found in 72% of the study population, with 2.3% affected by severe tail lesions (scores ≥ 3). Pleurisy (13.7%) followed by pneumonia (10.4%) showed the highest disease prevalence, whereas ascariasis showed the greatest variation between batches (0 to 75%). Tail lesion score was associated with condemnations due to pleurisy, pneumonia and pleuropneumonia ($P \leq 0.05$) at a batch level but not at an individual level. VI shift was associated with condemnations for pneumonia, pleuropneumonia and pericarditis ($P \leq 0.05$) at a carcass level, and with pneumonia at a batch level. Sex was not associated with viscera condemnation but males were more likely to be affected by tail lesions ($P \leq 0.05$).
Conclusion: The relationship between overall tail lesion score and condemnations due to lung disease supports the relationship between poor health and poor welfare of pigs on farms and reinforces the potential use of post mortem meat inspection as a health and welfare diagnostic tool. The association at batch but not at individual level suggests shared risk factors for health and welfare problems affecting pig herds. The association with VI shift suggests that the identification and classification of certain diseases is inconsistent between VI.

Disclosure of Interest: None Declared

Keywords: meat inspection, respiratory disease, tail biting

Herd Health Management and Economy

PO-PT2-062

Effects of energy supplementation to neonatal low birth weight piglets on mortality, daily weight gain, weaning weight and colostrum intake

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Introduction: Pre-weaning piglet mortality is an important economic and welfare problem in commercial pig industry and is mainly due to an energy deficit. Management strategies, such as energy supplementation to neonatal piglets, are needed to reduce pre-weaning mortality. Energy supplements may provide directly energy to neonatal piglets as well as improve their colostrum intake. In practice, energy supplementation can be easily implemented in the farrowing management. Therefore, the present study investigated the effect of a commercial energy supplement (Vigorol®) to neonatal low birth weight piglets on mortality, daily weight gain, weaning weight as well as the effect on colostrum intake.
Materials and Methods: Colostrum intake was calculated by the mechanistic model developed by Theil et al. (2014). In the treatment group, 165 low birth weight (**LBW**) piglets (≤ 1.2 kg) out of 332 total live born piglets from 25 litters were orally supplemented at birth and 8 to 12 h after birth. In the control group, 154 LBW piglets out of 349 total live born piglets of 25 litters were not supplemented.
Results: In general, mortality was lower in the treatment than in the control group at day 3 ($p = 0.02$), day 7 ($p = 0.03$) and day 21 ($p = 0.02$). The mortality of LBW piglets was lower in the treatment than in the control group at day 7 ($p = 0.03$) and day 21 ($p = 0.02$). The mortality of very low birth weight **(VLBW)** piglets (< 1.0 kg) was lower in the treatment than in the control group at day 3 ($p = 0.02$) and day 7 ($p = 0.02$). There was no difference in mortality between the groups for the normal birth weight (**NBW**) piglets (> 1.2 kg). The overall daily weight gain and weaning weight were lower in the treatment than in the control group ($p < 0.001$). Regarding the (V)LBW and NBW piglets, there was no difference for daily weight gain nor weaning weight between both groups. Colostrum intake per piglet, per LBW, VLBW or NBW piglet did not differ between the treatment and control group ($p > 0.05$). Colostrum intake by the litter was numerically lower ($p > 0.05$), but more uniform ($p > 0.05$) in the treatment than in the control group.
Conclusion: This study clearly demonstrated that energy supplementation to neonatal LBW piglets is a practical measure to reduce (LBW) piglet mortality. As piglet mortality is an increasing welfare concern in addition to an economic problem, pig producers can implement this measure in their farrowing management to increase pre-weaning survival.

Disclosure of Interest: None Declared

Keywords: colostrum , energy supplementation, mortality

Herd Health Management and Economy

PO-PT2-063

Influence of farm-specific parameters on therapy indices, average daily dosage and defined daily dose on fattening farms in South West Germany

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Introduction: The therapy indices, the average daily dosage (ADD100) and the defined daily dose (NADD) have been calculated for 72 fattening farms in South West Germany. The objective of this study was to compare different methods for measuring the use of antibiotics used in Germany, the Netherlands and Denmark. Farms with a high antibiotic usage should have high results in every calculation model. Furthermore, the influence of several defined farm parameters regarding the extent of antibiotic usage was evaluated with the intent to develop an action plan.
Materials and Methods: Records on delivery and application of drugs were evaluated from 72 fattening farms in South West Germany. This information plus some data of the internet platform vetidata® were used to calculate the therapy indices as well as the ADD100, and NADD values. For better evaluation, the investigated farms were ranked according to their ADD100, NADD and therapy indices. Farm parameters (operation system, herd size, soil conditions, cleaning and disinfection, type and stocking density, daily weight gain and duration of fattening period in days, age of the buildings, regional pig density and mortality) were collected by using a questionnaire. Their influence on the relative ADD100, NADD and therapy indices was evaluated using regression analysis. P-values smaller than p=0.05 were considered to be significant.
Results: A significant correlation (p<0.001) was found between the three different calculation methods from Denmark, the Netherlands and Germany. The 25% of farms with the highest values of the therapy indices, the ADD100 and the NADD were identical and will/are to be reprimanded in Germany, the Netherlands and Denmark.
Farm parameters which were correlated with an increased use of antibiotic agents were “exclusive fattening” (p<0.001), small herd size (p=0.02) and no all-in/all-out (p=0.02). Parameters which had no significant influence on the antibiotic usage were the following: soil condition, cleaning and disinfection, stocking density, daily weight gain and duration of fattening period in days, age of the buildings, regional pig density and mortality.
Conclusion: The study showed that the existing calculation models for measuring the use of antibiotics in Denmark, the Netherlands and Germany are comparable. Furthermore, some variable farm parameters (operation system, herd size, type of stocking) had a significant influence on the amount of antibiotic usage and should be included in an action plan.

Disclosure of Interest: None Declared

Keywords: ADD100, NADD, therapy indices

Herd Health Management and Economy

PO-PT2-064

Factors associated with high mortality of the piglets in farrowing units

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Introduction: High mortality in the pig industry is a welfare problem as well as it reduces the farmers income. The Danish Pig farmers have decided to reduce the mortality with 20 % before 2020 compared to the 2011 level. For each percent the mortality is reduced in the farrowing units the gross margin per sow per year is increased by 6 euro. The objective of this study was to provide knowledge of the differences in management, health, feeding- and housing conditions that impact on piglet mortality in the farrowing units.
Materials and Methods: The hypothesis was that identification of differences in management, health, feeding-, and housing conditions in herds with high and low mortality can identify risk factors for mortality of piglets in farrowing units.
The study was performed as a case-control study in farrowing herds with high or low total mortality (stillborn and preweaning mortality). Farrowing units with low mortality had a total mortality ≤ 20.3%, and farrowing units with high mortality had a total mortality ≥ 24.6%. Each farm was visited by a veterinarian and a comprehensive questionnaire was filled in during the herd visit.
The initial statistical univariate analysis was performed by logistic regression (Proc Logistic in SAS). Factors significant at P < 5% were considered for further analysis. Factors were selected by two criteria. There should be a plausible biological link to mortality and it should be possible to change the factor. To check for confounders, factors that were biologically plausible but hard to change were also included in the multivariate analysis. The results are presented as Odds Ratios (OR) for being a farrowing unit with high mortality. An OR > 1 indicates an increased risk and OR < 1 indicates a reduced risk.
Results: A total of 87 farrowing units, where 46 had high mortality and 41 had low mortality, were included in the study. The multivariate analysis showed that for each percent of sows with parity ≥ 7, the OR for being a farrowing unit with high mortality was 1.17. For each percent of sows treated for pain, the OR was 1.02. Herds where piglets were mingled before cross fostering had lower risk of being a farrowing unit with high mortality (OR 0.29). Herds with high pressure washing between farrowing batches and consistently opening of covered creep area by daily supervision also had reduced risk of having high farrowing unit mortality (OR 0.21 and OR 0.19).
Conclusion: This study indicates that the risk of being a farrowing unit with high mortality is associated with number of old sows, treatment for pain, cross fostering procedure, hygiene level (high pressure washing), and the level of supervision of the piglets.

Disclosure of Interest: None Declared

Keywords: Case control, Preweaning mortality, Risk factors

Poster Abstracts

Herd Health Management and Economy

PO-PT2-066

Factors associated with high mortality in nurseries

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Introduction: High mortality in the pig industry is a welfare problem as well as it reduces the farmers income. The Danish Pig farmers have decided to reduce the mortality with 20 % before 2020 compared to the 2011 level. For each percent the mortality is reduced in the nursery, the gross margin per produced 30 kg pig is increased by 0.33 euro. The objective of this study was to provide knowledge of the differences in management, health, feeding- and housing conditions that impact on mortality.
Materials and Methods: The hypothesis was that identification of differences in management, health, feeding-, and housing conditions in herds with high and low mortality can identify risk factors for high mortality rates in nurseries.
The study was performed as a case-control study in nurseries with high or low mortality. Nurseries with low mortality had a mortality ≤ 1.8%, and nurseries with high mortality had a mortality ≥ 3.5%. Each nursery was visited by a veterinarian and a comprehensive questionnaire was filled in during the herd visit. The initial statistical univariate analysis was performed by logistic regression (Proc Logistic in SAS). Factors significant at P < 5% were considered for further analysis. Factors were selected by two criteria. There should be a plausible biological link to mortality and it should be possible to change the factor. To check for confounders, factors that were biologically plausible but hard to change were also included in the multivariate analysis. The results are presented as Odds Ratios (OR) for being a nursery with high mortality. An OR > 1 indicates an increased risk and OR < 1 indicates a reduced risk.
Results: A total of 60 nurseries, where 24 had high mortality and 36 had low mortality, were included in the study. The multivariate analysis showed that herds with PRRS type2 had an Odds Ratio (OR) of 28 for being a nursery with high mortality compared to a herd without PRRS type 2. The OR was 5.9 for each percentage of pigs with umbilical hernia. Herds with low risk of mortality were characterized by collection of small pigs into one pen in the same section (OR 0.04), good biosecurity (OR 0.13), weighing pigs before entering the nursery (OR 0.01), and by dry feeding (OR 0.003).
Conclusion: This study indicates that the risk of being a nursery with high mortality is associated with feeding system, disease (PRRS and hernia), biosecurity, piglet weight and the handling of small piglets.

Disclosure of Interest: None Declared

Keywords: Case control, Nursery mortality, Risk factors

Herd Health Management and Economy

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Next generation management tools being developed for producer group specific and larger-scale (aggregate data) swine health monitoring applications

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Introduction: Streamlined systems that link diagnostic submissions and corresponding test results to spatiotemporal disease management tools are needed to enhance pork producers' ability to proficiently monitor, manage, control, and/or eliminate endemic diseases of significance across farms, production systems and regions. The primary objective our efforts have been to develop a scalable and broadly applicable streamlined system for linking veterinary diagnostic laboratory submissions, corresponding test results, attending veterinarian insight, and an interpreted health status of farm sites to a spatiotemporal disease management tool (Disease BioPortal®, University of California - Davis) for use in area-regional, veterinary clinic, or production system swine health monitoring and control initiatives. A secondary objective has been to create a working example where by these web-based tools are used to collate diagnostic data (identified only to the state of origin) from VDLs across the US for aggregate, time, and space diagnostic trend analysis.
Materials and Methods: Pilot projects were utilized to enable access to the premises-specific information and corresponding flow of diagnostic information that were needed to evaluate and trouble-shoot the performance and functionality of the systems and web-based animal health information management tools being developed. Collaborations within the USDA National Animal Health Laboratory Network were forged to link the PEDV and PDCoV PCR results being reported to the USDA from VDLs located throughout the US to a dynamic web-based dashboard in Disease BioPortal®.
Results: These efforts have led to the development of a suite of complementary methodologies and web-based tools (Animal Health Information Management Network) that will provide producers a new system for monitoring and maintaining the health status of swine farms in their practice, region, or production system over time. The tools and systems of inter-laboratory connectivity being further developed in the Animal Health Information Management Network were used to establish a web-based dashboard of time and space sensitive graphics in Disease BioPortal® that depict the national and state level trends in case level PEDV and PDCoV PCR test results observed in case submissions made to VDLs and reported to the USDA.
Conclusion: Collectively, this suite of novel methods, web-based tools, and systems of inter-laboratory connectivity being advanced and further developed hold promise for helping create a step-wise improvement for both producer group specific and larger-scale (aggregate data) swine health monitoring applications.

Disclosure of Interest: None Declared

Keywords: health status, information, management

Herd Health Management and Economy

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MONITORING OF RESPIRATORY DISEASES BY SLAUGHTER EXAMINATION OF PNEUMONIA AND PLEURISY

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Introduction: Respiratory diseases (RD) are one of the most important disease conditions in intensive pig production systems worldwide. Slaughterhouse examinations of macroscopic lung lesions are a reliable method for assessing the effect of RD at herd level. The aim of this study was to investigate the prevalence and severity of pneumonia and pleurisy in Belgium and to create a tool which may support diagnosis and monitoring of RD, as well as benchmarking between pig herds.
Materials and Methods: Between January 2012 and December 2015, 113 batches from 73 commercial pig herds were selected and included in the study. Inclusion criteria were limited to herds that sent to the slaughterhouse more than 40 pigs/batch, either with or without clinical history of RD and independently of vaccination strategy against respiratory pathogens. Pneumonia was scored using BOLLO score (0-5). Prevalence and severity of pneumonia lesions were calculated for each herd. Pleurisy was scored using SPES score (0-4). Prevalence and severity of pleurisy were calculated for each herd. Based on these calculations, APP-index (APPi) was computed as the % of lungs with SPES score>1 multiplied by the average SPES score of all the lungs. All the slaughterhouse examinations were performed by 3 trained experts.
Results: In total, 20017 lungs were scored. On average, 177 pigs/batch were investigated [41 – 433]. All pig herds presented at least one pig affected by pneumonia and pleurisy, even without clinical signs of RD at herd level. The overall prevalence of pneumonia was 26.4%, whereas 7.2% were severe lesions (BOLLO>2). The average within-herd prevalence of pneumonia was 29.0% [95% Confidence Interval (25.3-32.6%)]. Quartiles for within-herd prevalence of pneumonia were 12.0% (Q1), 25.8% (Q2) and 45.5% (Q3). The overall prevalence of pleurisy was 40.5%, whereas 21.3% were severe lesions associated with *Actinobacillus pleuropneumoniae* infections (SPES>1). The average within-herd prevalence of pleurisy (SPES>1) was 21.9%. The average within-herd APPi was 0.63 [95%CI (0.53-0.73)]. Quartiles for within-herd APPi were 0.17 (Q1), 0.44 (Q2) and 1.02 (Q3).
Conclusion: This study revealed a high prevalence of pneumonia and pleurisy in Belgium. The evaluation of lung lesions at slaughter supports diagnosis of RD. The interquartile range for pneumonia and APPi provided an effective tool for herd monitoring and classification. This classification can be used to evaluate the evolution of RD after implementation of control measures (vaccination, management and housing), as well as to perform benchmarking between different herds or production systems.

Disclosure of Interest: None Declared

Keywords: lung lesions, monitoring, Swine Respiratory Disease

Herd Health Management and Economy

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A novel model for estimating the number of consecutive negative samplings required to meet user-specified confidence of disease elimination.

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Introduction: Disease elimination protocols are expected to result in the targeted disease agent being no longer present in the animal population and flow. To judge the success of elimination efforts, it is common to conduct recurring sampling over time from targeted animal cohorts. Whereas sample size determination methods for disease detection from single samplings are generally well understood, the importance of recurring sampling in judging successful disease elimination is poorly understood and executed. As it relates to sampling, confidence in the success of elimination protocols is influenced not only by sample size for each sampling but also by repeated sampling. Production systems continuously produce new cohorts of animals (e.g., groups of weaned pigs) that grow through sequential production phases, typically being moved from one physical location to another location. Opportunities for measuring disease elimination success occur as each new cohort of animals exits a location and moves downstream to the next. To support this aspect of appropriate sampling protocols for judging disease elimination, a methodology, algorithm and model were developed to incorporate both aspects of confidence – sample size and number of samplings.
Materials and Methods: Basic model user-defined input variables are: animal population size, animal prevalence, sample size (per sampling), assay sensitivity/specificity, number of sequential samplings and number of simulation runs. The model accommodates selection of: sampling with or without replacement, and fixed or stochastically-generated prevalence. To test the model a population of 1000 animals and sample sizes of 15, 30, 60 and 90 were used to generate sets of data at animal prevalence levels of 1%, 3%, 5% and 10%. A total of 100 model runs of 20 consecutive samplings per run were generated for each prevalence level. All sets of runs were generated using sampling without replacement and stochastically-generated prevalence. The detection threshold of interest was the sampling at which $\geq$95% of model runs were detected as positive for the specified prevalence level.
Results: At the 1% prevalence level, for sample sizes 15, 30, 60 and 90 the 95% detection threshold was achieved at the 15th, 10th, 6th and 3rd samplings, respectively.
Conclusion: This novel sampling model can be used to dynamically estimate the appropriate number of consecutive samplings at given sample sizes collect for use in judging the success or failure of disease elimination protocols, as well as generate tables to be used as references for disease detection sampling that represent the role of recurring samplings.

Disclosure of Interest: None Declared

Keywords: None

Poster Abstracts

Herd Health Management and Economy

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Endemic respiratory disease antibody monitoring – field study

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Introduction: Antibody monitoring of endemic respiratory disease pathogens in swine populations is a diagnostic tool that facilitates herd health management. Routine monitoring with oral fluid or serum enables early detection of seroconversion to pathogens in contemporary pig herds, confirms the continued absence of exposure to particular pathogens, and is an excellent tool to ensure vaccination compliance and proper vaccination timing.
Materials and Methods: Two on-site growing gilt units and a commercial wean-to-finish barn collected biweekly pen-based oral fluid samples to monitor antibodies to PRRSV (IDEXX PRRS OF), and monthly serum samples to monitor serologic antibodies to PRRSV (IDEXX PRRS X3), *M. hyo* (IDEXX *M. hyo*) and influenza A virus (IAV) (IDEXX Swine Influenza). Oral fluids (OF) were collected from the same pens at each time point, and sera were collected monthly from a subset of animals in these pens.
Results: OF was collected 13 times, and sera, 7 times, throughout the W-F growing period. Sera remained negative for PRRSV and M. hyo antibodies (S/P <0.40), OF remained negative for PRRS antibodies (S/P <0.40), and IAV serology showed maternal antibodies declining by 12 weeks of age, thereafter remaining negative (S/N > 0.60). GDU 1 and 2 used similar sampling intervals to the w-f site, for 2 groups of gilts each. In GDU 1, gilts were positive for maternal antibodies (Mab) to PRRSV which declined, but then seroconversion was detected by OF at 10wks of age. Similarly, GDU 1 weaned pigs were negative to *M hyo*, but by 10wks of age there was evidence of seroconversion. IAV Mabs were identified in all GDU 1 weaned gilts, but these declined and no further IAV seroconversion took place. In GDU 2 PRRS and M. hyo antibody status began and remained negative in both groups of gilts. However, though IAV Mabs declined in these gilts around 10wks of age, 1 group became exposed to IAV and began seroconverting (S/N ≤ 0.60) at 14wks of age.
Conclusion: Endemic disease antibody monitoring is an excellent tool for confirming proper implementation of exposure programs. It provides useful insight into the immune status of growing females acclimated in GDUs or isolation prior to sow farm entry, and gives confidence in the continued freedom from exposure to key production impacting pathogens.

Disclosure of Interest: None Declared

Keywords: endemic disease, antibody monitoring

Herd Health Management and Economy

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Influence of sow parity and birth weight on finishing pig performance

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Introduction: Selection on increased litter size results in lowering the average birth weight with 35 gram per additional piglet per litter. In addition it is known that offspring of gilts are 170 grams lower in birth weight compared to elder, 2nd-5th parity sows. To gain more insight in the effect of birth weights and the effect of sow parity on the performance of their offspring, the piglets were followed from birth until slaughter.
Materials and Methods: From a previous trial focusing on colostrum uptake of piglets, sow characteristics (parity, weight at d107 of gestation) were known. The piglets (n=815; Topigs 20 x Tempo; boars and gilts) were followed from birth until slaughter in the period December 2012 – July 2013. Piglets were eartagged using chip containing notches. Piglets were weighed individually at several time points at the farrowing farm (birth, d7, d24 at weaning, d14 and d35 after weaning). At an age of 9 weeks (20 kg) pigs were transported to the finishing site. Finishing pigs were weighed at the start of finishing and 4, 8 and 12 weeks of finishing. Pigs were sent to slaughter at 115 kg weight, and individual carcass information was collected (muscle thickness, backfat thickness, carcass weight and lean meat percentage). Statistics were done with SAS (generalized linear mixed models - PROC GLIMMIX).
Results: Offspring of gilts had a higher market age compared to offspring of elder parity (2-7) sows (183 vs 179 days p<0.01) and a lower muscle (59.9 vs 61.9 mm p<0.05) and back fat (12.4 vs 13.4 mm p<0.05) thickness. Sow weight at 107 days of gestation influenced the number of stillborn piglets and market age of offspring. The heaviest 25% sows (>268 kg) had a higher percentage of still born piglets compared to the other sows (6.08 % vs 2.84%). Offspring of the 2nd quartile sows (213-245 kg) had the highest colostrum production (327 vs 274 gram/piglet; p<0.05), in combination with the highest growth during the finishing period (795 vs 773 gram/day (P<0.05). Based on birth weight, pigs were categorized in 4 quartile groups (1st <1.1 kg; 2nd 1.1-1.28 kg; 3rd 1.28-1.46 kg; 4th >1.46 kg). Birth weight influenced growth after weaning (341, 363, 384, 392 gram/day respectively p<0.01) and market age (184, 182, 180, 177 days respectively; p<0.01).
Conclusion: Low birth weight influences finishing pig performance reducing ADG. First parity sows and heavy sows limit the growth potential of their offspring. A balanced parity distribution in combination with a homogenous bodyweight distribution of the sows is important for an optimal performance of the finishing pigs. This will also aid in more weaned piglets by reducing the number of still born piglets and by increasing the amount of produced colostrum.

Disclosure of Interest: None Declared

Keywords: birth weight, finishing pigs, sow parity

Herd Health Management and Economy

PO-PT2-086

Survey of Austrian pig practitioners on methods and timing of euthanasia in pigs

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Introduction: Veterinary practitioners and pig farmers are often faced with the decision on how to deal with ill, injured or wasting animals, wait and treat them or euthanize them. In addition, there is the question of "who" and "how". In the European Union there are no uniform regulations or action advices by now. In an anonymous survey, pig veterinarians were asked for their assessment and personal approach to impose a status quo in Austria.
Materials and Methods: In 2015, as part of a swine practitioner conference in Austria, approximately 80 % of the Austrian swine practitioners (22 female and 33 male) were interviewed anonymously by Clicker System (Interactive Presenter, company Dolphin Interactive).
Results: 78 % of practitioners are called by their farmers to euthanize pigs at least occasionally, 20% of them even regularly. While male colleagues decide faster to euthanize, female swine practitioners rather try one last attempt of treatment. Women prefer to euthanize pigs themselves, while men seem to leave the emergency killing rather to the farmer. Nearly 84 % of the respondent veterinarians euthanize pigs are using a combined preparation of tetracaine, Mebezoniumiodid and embutramide (T61®- solution for injection of animals, Hoechst Roussel Vet GmbH, Wiesbaden); however, 38 % of vets administer it without preceding anaesthesia, which is illegal. At least, 11 % of the practitioners (all older than 45 years) euthanize pigs using captive bolt or blunt trauma to the head and only half of them bleed the animals afterwards. 60 % of the practitioners, regardless of age or sex, believe that further training of farmers in euthanasia is not necessary. Average costs of euthanasia of nursery piglets by the veterinarian are 25 €, euthanasia of a sow costs between 10 and 50 €, regardless of the method. 55 % of the vets guess that the farmers kill the piglets by blunt trauma, but only 29 % of veterinarians estimate that piglets are bled afterwards. 87 % of practitioners, especially female colleagues, believe that farmers need better trained on emergency killings. Nevertheless, they prefer leaving the emergency killing of piglets, weaners and fatteners rather to the farmer. Working cattle guns or battle shooting tools, however, are only found in estimated 50 % of farms.
Conclusion: A standardization of methods and assistance in the form of a decision tree for an emergency killing as well as training for farmers on the correct procedure of killing pigs from different age groups is absolutely necessary. An increased involvement of the attending herd veterinarian in the decision-making process of the farmer would be welcome.

Disclosure of Interest: None Declared

Keywords: emergency killing

Herd Health Management and Economy

PO-PT2-087

Evolution of antibiotic consumption in an "antibiotic-free" porcine production chain

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Introduction: Antibiotic resistance and reduction of antibiotic use are main issues in pork production. In collaboration with vet practicioners in charge of health management, a french producer organization initiated an « antibiotic-free » production chain in June 2014. Farmers that signed the adhesion contract had to raise pigs without administering antibiotics from birth to slaughter.
Materials and Methods: The 29 farms that were part of this production chain were included in the study. Those farms are located in the Normandy region, West of France. The producer organization provided the technical parameters to estimate animal populations to which antibiotics could have been given. Vet practicioners gave access to the lists of drug sales. A questionnaire was performed to precisely the antibiotic use pattern in each farm. Two different six-month periods were selected for the data collection: August 1st, 2013 to January 31th, 2014 (P1) and November 1st, 2014 to April 30th, 2015 (P2). These periods were situated before (P1) and after (P2) the inclusion in the antibiotic-free protocol respectively. The use of antibiotics is expressed in weight, nDD/animal (number of Daily Dose) and nCD/animal (number of Course Dose).
Results: Twenty-five farmers accepted to participate and the herds of the sample were similar to the french pig herds on average in terms of size and technical performances. The total amount of anitibiotics used in the farms of the sample decreased by 64.2% between P1 and P2. In five farms, antibiotic consumption increased because of occurrence of acute health disorders. All physiological stages underwent a decrease of both nCD and nDD/animal values between P1 and P2. For weaners, nDD/animal and nCD/animal decreased by 85 and 79% respectively. The oral route was predominant in P1 but this administration way exhibited the most important decrease. The sharp decline in the consumption of oral antibiotics indicates that there was no substitution of medicated feed by antibiotics *via* drinking water. The number of treatments by injections slightly increased between the two periods. In our sample, antibiotic use could be significantly reduced for weaners whereas and it was more difficult during the lactating period partly because of arthritis.
Conclusion: The establishment of an antibiotic-free production chain has led to a significant reduction in the use of antibiotics in most farms, except when disease outbreaks occured. It should be noted however that this producer organization benefits from a favourable sanitary status resulting from a dynamic health policy for several years (all herds are PRRS free and some are also *Mycoplasma hyopneumoniae* free).

Disclosure of Interest: None Declared

Keywords: antibiotic free, antibiotic reduction

Poster Abstracts

Herd Health Management and Economy

PO-PT2-090

Cost of production diseases to pig farms

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Introduction: Production diseases usually originate from a complex interaction of pathogens, animal genetics and environment. They compromise animal health and welfare, can reduce product quality and increase environmental footprint of pig production and the use of antimicrobials. Production diseases impact farm economy and can result in loss of revenues and extra production costs. However, very limited synthesis on economic impacts of production diseases is available. The goal of this study is to review the costs of production diseases in pigs.
Materials and Methods: An extensive review of published studies was undertaken. Studies published during 1995-2015 were searched in electronic databases (Web of Science, Scopus, Google scholar, International Veterinary Information Service, the websites of FAO, OIE, British Pork Executive, Danish Meat Association, the PigSite, Cordis). The search resulted in 130 publications for review. These studies were field trials, experiments, modeling studies or reviews. Prior to analyzing the data, cost estimates reported in the studies were converted to per animal-basis by using study or country-specific data, deflated to year 2014 and converted to euros.
Results: Reviewed studies showed great case-by-case variation in the costs of production diseases. Respiratory diseases are an economically important issue in pig production. According to the review, economic losses due to porcine respiratory disease complex (including Mycoplasma hyopneumoniae, Actinobacillus pleuropneumoniae and other associated pathogens) are about €6.8 per fattening pig produced by an affected herd. Depending on pathogen and case, these losses ranged from €2 to €19 per fattening pig produced. Realistically, several diseases can occur in the herd simultaneously, pushing up total costs above these estimates.
The impacts of mortality per individual dead pig can be high, but only dead animals result in mortality losses. In the studies analyzed, the reduction in returns due to pre-weaning mortality was between €12 and €23 per litter, and due to post-weaning mortality between €2 and €4 per pig.
The costs of mastitis or the complex syndrome 'Mastitis, Metritis and Agalactia' can range up to €95 per affected sow. In the most severe cases the impacts can be even larger. The costs of lameness were available on a 'per lame pig' basis. In sows, the costs ranged from €145 to €180 per lame sow.
Conclusion: Production diseases, in particular, respiratory diseases can cause substantial losses. The losses can vary from farm to farm and case by case. The review suggests that the current literature on economic costs of production diseases is limited.
This work was conducted under the EU-funded PROHEALTH project.

Disclosure of Interest: None Declared

Keywords: Economic loss, Pigs, Production diseases

Herd Health Management and Economy

PO-PT2-097

POST-FARROWING TREATMENT OF SOWS WITH ORAL MELOXICAM (METACAM®) OR INYECTABLE GENERIC MELOXICAM ON THE PREWEANING WEIGHT GAIN IN SUBCLINICAL MMA

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Introduction: Mastitis-metritis-agalactia (MMA) is a complex syndrome in which hypogalactia or agalactia occurs in a clinical or subclinical way within the first hours postfarrowing (1). In previous field studies, Oral Metacam® 0.4 mg/kg b.w (Boehringer Ingelheim Vetmedica GmbH) has proven to be effective in the treatment of MMA, allowing significant reduction of the mortality rate and improvement of the weight gain of piglets during the lactation period (2,3,4,5). The aim of this study was to compare the convenience and the efficacy of the use of a single administration of Metacam® 15mg/ml oral suspension for pigs (Oral Metacam®) versus other generic injectable meloxicam 20mg/ml on sows in piglet performance.
Materials and Methods: The field trial was conducted on one farrow-to-wean farm (1300 sows) located in the Northeast of Spain. Severe agalactia was not observed during or after the trial. Overall, 127 sows were randomly allocated the day of farrowing (d0) to two homogeneous groups and blocked by parity, number of piglet per sow and weight at birth. One group (n=63) was given 0.4 mg/kg b.w of Oral Metacam® (Boehringer Ingelheim Vetmedica GmbH) directly into the mouth on the day of farrowing. The other group (n=64) was treated with generic meloxicam 0.4 mg/kg b.w. intramuscular. The efficacy of the treatment was evaluated by measuring suckling piglets mortality by litter, the number piglets weaned per sow and the litter weight gain. Piglets of the same litter were weight together at day 0 and at weaning and the averages were compared. The litter was the experimental unit. Data were analyzed by ANOVA test procedures using SPSS v.15.0 software (SPSS Inc., Chicago, IL, USA).
Results: The piglet loss by litter was numerically lower in the Oral Metacam® group (2.04vs2.74). Total piglet weaned per sow (figure 1) was significantly higher in the Oral Metacam® group compared to generic meloxicam group (9.92vs.9.19). In addition, litters from sows treated with Metacam showed a significantly higher weight gain compared to the litters from sows treated with generic product (31,24kg. vs 26,05 kg.).
Conclusion: Regarding efficacy, Oral Metacam® treatment in sows significantly increased the litter weight gain from during lactation compared to generic meloxicam. Furthermore, piglet mortality rate was reduced so the number of piglets weaned per sow was statistically increased. This Metacam oral presentation reduces the number of injections during the farrowing period and result in additional benefits for sow welfare.

Disclosure of Interest: None Declared

Keywords: Oral Metacam, piglet performance

Herd Health Management and Economy

PO-PT2-103

Critical success factors of PRDC management in Central-Europe

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Introduction: Cooperation and joint technical knowledge of vets and farm managers (experts) are needed for effective pig farming. They have a real influence on production indices and on manifestation and predisposing factors of Porcine Respiratory Disease Complex (PRDC). At the same time the ineffective farm management is responsible for imprudent overuse of antibiotics and economic losses. In this study we surveyed Hungarian, Czech and Slovak swine farm experts' opinion on critical farm management factors of PRDC.
Materials and Methods: From February to May 2014 63 vets and 38 farm managers were personally questioned about theoretical (TH) and practical (PR) key success factors and their emergency needs (EN) in PRDC management by using the ResPig™ questionnaire. The 101 swine farm experts were from 63 farms (66,500 sows) located in Hungary, 11 (14,050) in the Czech Republic and 1 (2,000) in Slovakia, respectively. Vets and farm managers had to select the 10 most important PRDC prevention and control management factors out of 30 in terms of building a new farm (TH) and the operation of the farms that they work on (PR). Furthermore, they had to select and score on a 100 point scale the most critical PRDC management factors in their herds' future operation (EN).
Results: According the 101 respondents the 10 most critical success factors in PRDC when a new farm is built are as follows: Herd Health Security–HHS (74% of the respondents), All-In-All-Out–AIAO (70%), Good Vet Practice-GVP (70%), Staff-S (67%), Feed Quality–FQ (67%), Management–M (60%), Farm Isolation–FI (60%), Low Farm Density–LFD (60%), Low Disposal Rate-LDR (mortality, culling) (60%), Stocking Density–SD (55%). In operation of their farms the 10 most important factors are: AIAO (100%), S (100%), GVP (86%), FQ (86%), M (86%), HHS (71%), Hygiene Level–HL (57%), Feed Safety – FS (43%), Owner Attitude – OA (43%), LFD (29%). In a 100 point scale the 10 most critical factors are: LFD 65 points on average, AIAO 64, FS 57, FQ 55, HL 53, GVP 50, HHS 49, S 49, M 49 and OA 48.
Conclusion: HHS, AIAO, GVP, S, LFD, FQ and M were identified as critical factors in PRDC management in every survey and FS, HL and OA were selected as key elements of the operation. LDR was only selected in one survey as production index. Feed conversation ratio, average daily gain and number of pigs marketed were never identified as key factors. The findings of the study revealed that the lack of effective farm management and the relating prevalent, recurring, operational problems on the farms (weak HHS, lack of AIAO, unsure FQ and poor HL) have the most detrimental effect on the PRDC status on Central-European pig farms.

Disclosure of Interest: None Declared

Keywords: PRDC–management, swine, vets, farm managers

Herd Health Management and Economy

PO-PT2-105

A study to evaluate repeatability of feeding time of sows in large group gestations with ESF systems.

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Introduction: Animal welfare, has been traditionally evaluated by measuring level of aggression and stress parameters. However, feeding behavior can also provide a valuable indication of comfort or stress for gestating sows in large groups, when fed with ESF systems.
Materials and Methods: The study was conducted at Albesa-Ramadera a 3300 sow, Site 1 farm, based in Catalonia, Spain. The farm has large group gestation (128 to 175 sows per group) and utilizes ESF. (Compident 7®, Schauer Agrotronic GmbH). Nulliparous sows are placed separated in dynamic pens, while all other multiparous sows, (parities 2-7) are placed in larger dynamic groups.
By analyzing one week data; feeding time repeatability was considered a potentially good indicator of group feeding stability.
Four dynamic pens were considered in this study; Pen 2 and 6 were multiparous pens with 172-175 sows in each, respectively. Pen 7 and 8, were nulliparous pens with, 128 and 140 sows in each, respectively.
A parameter called; Percentage of time coincidence (PTC), was created to evaluate with what deviation from the average entry time the sow eats one day, as compared with her average entry time during a one week period.
Results: PTC=1, means that the sow eat every day at the same time. PTC=0,99-0,8 means that these sows eat every day within a ± 90 minutes deviation, from the average eating time. PTC= less than 0,8, means that these sows had a large variation of feeding time within the period of the study.
When classifying sows according to their PTC, it can be seen that in multiparous pens, 62 to 73% of sows eat more or less at the same time of the day, (±90 minutes every day), while the rest is less consistent in their entry time.
It can also be seen that in nulliparous pens, 51 to 75% of sows eat more or less at the same time of the day, (±90 minutes every day), while the rest is less consistent in their entry time.
Conclusion: PTC tends to be higher in more stable groups, (longer time since last entry of sows) and in groups where sows have had adequate training.
The large difference in between groups of same type (Multiparous, or Nulliparous), is related to the last entry of sows into the pen. In pen 6 and 8, animals had been added during the week of the study.
Stable groups independent of their parity have a large percentage (73-75%) of sows eating within ±90 minutes, of their previous feed.
The parameters measured could be considered useful to evaluate normal or abnormal feeding behaviors especially within farm comparisons.

Disclosure of Interest: None Declared

Keywords: Electronic sow feeding behaviour

Poster Abstracts

Herd Health Management and Economy

PO-PT2-106

The development of sow mortality in Denmark since 1990

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Introduction: During the last 25 years, the sow mortality in Denmark (DK) has moved from a low level back in 1990 to a very high level in 2007. It subsequently dropped to an acceptable level in 2015. For years, sow mortality was neglected by farmers and pig advisors despite the significant losses incurred. However, since 2007 sow mortality rates have been an issue at the monthly herd health visit and this has paid off in most herds.
Materials and Methods: The rising sow mortality in the period from 1990 to 2007 is attributed not to one but many reasons such as the legislation on restricted transportation of slaughter sows, new breeding goals for litter size, the introduction of loose housing during gestation, and animal welfare legislation in relation to shoulder ulcers.
In the beginning of 2000s, SEGES Pig Research Centre initiated a two-year survey in 37 sow herds on culling reasons. This information formed the basis of the goal set in 2007 that by 2013 max 11.5% of the sows in DK were dispatched to the rendering plants. This goal was met by the end of 2015 through dedicated research and an annual letter to farmers reminding them of their sow mortality rates. In 2005, sow longevity was incorporated in the Danish breeding system and this also contributed to reaching the goal.
Results: According to official reports submitted to the Danish Food Advisory Service by the Danish rendering plants, the number of sow carcases processed every year has dropped from 174,000 in 2007 to 117,000 in 2015. In this period, the number of sows in Danish herds has decreased from 1,158,000 to 1,034,000. This means that the percentage of all sows in DK dispatched to rendering plants has gone down from 15.3 to 11.4.
Sow mortality is calculated differently from the above method in sow herd management programmes around the world. However, using comparable sow mortality figures, Denmark is still in the lead. According to InterPIG data, sow mortality in Denmark was 4.5 percentage points higher than in the Netherlands and 5.9 percentage points higher than in the UK in 2014.
Conclusion: In 2012, sow mortality recorded in 1,620 sow herds with more than 250 sows varied from 2.8% to 40.2%. The Danish Food Advisory Service subsequently considered introducing a yellow card scheme to ensure a decrease in sow mortality. This has not yet been implemented and if we look at the current trend at national level it seems reasonable not to do so.

SEGES Pig Research Centre has now set a new goal: by the end of 2018, the number of Danish sows dispatched to rendering plants must not exceed 9%.

Disclosure of Interest: None Declared

Keywords: development, mortality, sows

Herd Health Management and Economy

PO-PT2-107

Characteristic findings in piglets dying from trauma

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Introduction: Preweaning mortality of piglets remains a welfare as well as an economic concern in commercial swine herds. Post mortem examination can give insight in specific preventive measures.
Materials and Methods: A thorough investigation of preweaning piglet mortality was conducted in 14 loose housed Norwegian piglet producing herds. All dead piglets from one batch of sows in each herd, in total 1216 piglets from 378 sows, were collected for necropsy. Death due to trauma was defined as a piglet with external and/or internal lacerations and/or fractures.
Results: Preweaning mortality, excluding the stillborns, was 15.3%. The weaning age was on average 33 days, and the oldest piglet that died was 32 days old. Of the 1200 piglets examined, 347 died of trauma (28.9 %). Among these piglets, almost 70 % had a full stomach, and nearly 25 % had an empty stomach. Additionally, 27 % of the piglets that died of trauma also had signs of anemia. Piglets died of trauma at day 2 after birth. However, as many as 158 piglets (45.5%) died after day 3. Average weight at death was almost 1.5 kg, ranging from 406 g to 4.7 kg. Considering the weight distribution, surprisingly heavy piglets died due to trauma. This was also reflected in a high Body mass index at death (average of 21 kg/m2).
Conclusion: The major cause of preweaning mortality of piglets in this study was trauma, mainly due to crushing. Compared to our finding of 28.9 % piglets dying from trauma, others have reported as many as 80 % of dead piglets having trauma as the cause of death. This may indicate that loose housing is not as risky as previously suggested. However, approximately half of the piglets were older than three days, a finding that call for attention on preventive measures. Our next step will be to investigate risk factors for trauma in piglets in our loose housed pens.

Disclosure of Interest: None Declared

Keywords: piglets, post mortem examination, Preweaning mortality

Herd Health Management and Economy

PO-PT2-109

Erysipela outbreak in young boars and the economic impact to a boar stud

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Introduction: *Erysipelothrix rhusiopathiae* is a Gram positive bacteria associated with septicemic infections in swine, that in acute cases can lead to a severe clinical presentation of high fever, lack of appetite, arthritis and endocarditis, and even death. This study aims to demonstrate the economic impact of an outbreak of Erysipela in young boars later introduced to a boar stud.
Materials and Methods: After the occurrence, treatment and recovery of an Erysipela case during the late finishing phase, a batch of 50 young boars were introduced to a boar stud and monitored during two months to analyze the impact of the outbreak in their semen quality and production rates. Semen volume, concentration and number of doses were compared between two groups, Ery (n=50; 272 collections) and Normal (n=54; 222 collections). Also, the percentage of collections trashed due to semen quality and percentage of boars that refused to mount the dummy were recorded.

Results: There was statistical difference ($p<0.05$) between the two groups only in semen concentration and number of doses, not in semen volume. Ery group showed 0.368 million sperm cells and 19.3 doses per collection, a difference of less 6.4 doses/collection per boar when compared to the Normal group, that in a year period totals 21 632 doses. Also, 18.8% of collections were trashed and 12.9% of boars refused to mount the dummy, in opposite to the Normal group that showed 6% and 0.9%, respectively.
Conclusion: It is well known that septicemic infections and high fever can negatively impact the semen quality, and the demonstrated results show the amount of opportunity loss due to an Erysipela outbreak, even after the complete clinical recovery. In some boars, there was a decrease in semen quality and also 12.8% more collections were completely trashed. Also 12% more boars refused to mount the dummy, which can be related to an arthritis, regarding the septicemic infection and joint lesion. Besides the opportunity loss of the production of semen doses, there are still costs to house sub productive males. To achieve the maximum production potential of the boar stud, it would be necessary to cull these boars and replace them.

Disclosure of Interest: None Declared

Keywords: Erysipelothrix rhusiopathiae, fever, Semen quality

Herd Health Management and Economy

PO-PT2-110

Claw lesions in neonatal piglets: a case study

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Introduction: Claw lesions in piglets are common. The neonatal claw is sensible for factors such as floor temperature and roughness, and possible residuals from disinfectants. Also selenium (Se) intoxication and ergot alkaloids have been related to claw lesions. The present case describes the occurrence of hemorrhagic claw lesions in neonatal piglets of a commercial farrow-to-finish pig herd in Flanders.
Materials and Methods: The herd consisted of 270 JSR sows in a 4-week batch system. Gestating sows were fed *ad libitum* and housed on straw bedding. Lesions had been observed for two months, they developed 1 to 2 days after birth and were present in nearly all litters. If present, lesions were seen in claws and dewclaws of all limbs. Lesions tended to be painful and started as small hemorrhages in the white line area of the claw, and became more extensive and eventually ulcerative after 1 to 2 weeks. Also necrotic tail lesions were found in some of these piglets. Neonatal piglets showed increased levels of diarrhea, arthritis and mortality (up to 20%). The differential diagnosis includes piglet- and environmental related factors as well as intoxications. Samples were taken for diagnosis: spleen for bacteriological analysis, liver for Se analysis and the hemorrhagic lesions for bacteriological and histological investigation. Feed was analyzed for mycotoxins prior to our first visit.
Results: Histological investigation revealed clear ulcerations of the coronary band's epidermis with invasion of round shaped bacteria and neutrophils. In mild lesions, bacterial colonization was combined with parakeratosis and scabbing. Bacterial invasion of hair follicles was also noticed. No histological abnormalities were found in the regions of the claw without macroscopic lesions. Bacteriological examination showed co-infection of *Staphylococcus spp.* and *Streptococcus suis* in all samples. Spleen samples were bacteriologically negative. Se concentration in liver-samples were within normal ranges (1.7 mg/kg). Ergot alkaloids were present in gestation and lactation feed, with the highest concentration during the gestation period (633.21 mg/kg; ref: 200 mg/kg).
Conclusion: The primary reason remains unclear, but we hypothesize that ergot alkaloids in sow feed affected the epidermal integrity of the neonatal claws, allowing the ubiquitous pathogenic bacteria to invade. However, when a mycotoxin binding and biotransforming feed additive was added to the feed during gestation and lactation, the problems were not solved.
Currently, piglets are treated with an antibiotic and a non-steroidal anti-inflammatory drug on day 1, the severity of the problem is significantly lower.

Disclosure of Interest: None Declared

Keywords: Claw lesions, Mycotoxins, Neonatal

Poster Abstracts

Herd Health Management and Economy

PO-PT2-120

IgY as a dietary supplement

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Introduction: They are several benefits of using IgY as a feed additive. Egg yolk or yolk plasma as a feed additive may improve the production results by two ways: 1) stimulation of growth rate through metabolism improvement and/or increasing feed intake; 2) stabilization and enhancing the gastrointestinal tract immune protection. The aim of the study was to compare two ways of IgY administration in the peri-weaning period (1 week before and 2 weeks after weaning) and to evaluate their influence on the production and health parameters in piglets.
Materials and Methods: Two experiments were carried out on the industrial farms. Exp.1(28 litters): into commercial feed there was added: 5% of the egg yolks from immunized hens (group II), 5% of egg yolk from non-immunized hens (group III), 1% addition of egg yolk plasma from immunized hens (group IV). Exp. 2 (21 litters): final mixes were balanced according to nutritional value with experimental additives: 1.5% of the egg yolk plasma from non-immunized hens egg yolk (group II), 1% of the egg yolk plasma from immunized hens (group III). Hens were vaccinated with Porcilis Porcoli DF. In both experiments group I (control) was fed with basic mix without additives. Health status (Ig, acute phase proteins, mortality) and production parameters (daily gains, feed consumption) were estimated. Blood was collect on: weaning day, 2 weeks later (finish of experimental mixes) and on the day of moving into fattening sector.
Results: Numerically higher daily gains were observed in experimental groups in both experiments. Daily gains during nursing period In Exp 1. were: 342; 380; 349; 379 g/d in I, II, III, IV groups, respectively. Differences were significantly different. In Exp. 2 daily gains were: 366; 367; 389 g/d in I, II and III groups respectively. In Exp.1 no differences occurred in morbidity and mortality rate between experimental and control piglets in contrast to the Exp.2 where they were higher in control group. Moreover it was found that the haptoglobin (Hp) concentration was the highest in both control groups two weeks after weaning. Also the highest differentiation were seen in those groups. -
Conclusion: No balancing crude protein and fat in Exp.1 probably lead to overfeeding during peri-weaning period in experimental groups, and therefore no significant health improvement was noted. The effects of different amount of plasma additive were seen in Exp. 2 when the feed was balanced to nutritional value. Lower Hp concentration in all experimental groups confirm immunoprotective effect of used additives.
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Disclosure of Interest: None Declared

Keywords: feed additive, IgY, piglets

Herd Health Management and Economy

PO-PT2-122

Prevalence of health conditions in grower pigs on Irish pig farms

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Introduction: On-farm pig health/welfare checks are labour and time consuming and therefore scores of carcass tail lesions during meat inspection are suggested as potential iceberg indicators. As part of a study to validate the use of carcass tail lesions in this way, we conducted health/welfare assessments on 31 Irish integrated pig farms using a modified Welfare Quality® protocol. Preliminary findings on the prevalence of the health conditions observed are presented.
Materials and Methods: Checks were conducted on 31 pig farms by observing 18 randomly selected pens of 1st (n=6) and 2nd (n=6) stage weaner and finisher (n=6) pigs per farm (July-Nov 2015). Pens were observed for 10 min and number of pigs with the following conditions was recorded: poor body condition (PBC), dead, sick (i.e. listless/laboured breathing), twisted snout, rectal prolapse, hernia, skin and neurological conditions, eye infections and lameness. These conditions were expressed as average percentage of pigs in a pen affected (mean ± SE) and were ranked to identify the three most common conditions in each production stage.
Results: In 1st stage pens the most commonly observed condition was PBC with 5.2 ± 0.4% of pigs affected (range: 0 – 30%), followed by pigs showing signs of sickness (1.7 ± 0.2%; range: 0 – 18.2%) and hernias (umbilical: 0.4 ± 0.1%; range: 0 – 18.2%; scrotal: 0.6 ± 0.1%; range: 0 – 7.1%). PBC was also the most commonly observed condition in 2nd stage pens (2.0 ± 0.2%; range: 0 – 12.5%) but with a lower proportion of pigs affected compared to the 1st stage. Hernias were the second most common condition (umbilical: 1.0 ± 0.2%; range: 0 – 15.3%; scrotal: 0.7 ± 0.1%; range: 0 – 16.7%) followed by lameness (1.0 ± 0.2%; range: 0 – 16.7%). In the finisher stage, hernias were the most commonly observed condition (umbilical: 1.8 ± 0.2%; range: 0 – 12.5%; scrotal: 0.4 ± 0.1%; range: 0 – 10.5%). Lameness was the second most commonly observed condition (1.3 ± 0.2%; range: 0 – 15.4%) and PBC the third (1.1 ± 0.2%; range: 0 – 12.5%).
Conclusion: Consistent with nutritional challenges associated with weaning, PBC was the most commonly observed condition in weaner pens. Weaning stress could also explain why sick pigs were only seen in the top three conditions for pigs in the 1st stage pens. As the pigs got older hernias and lameness became more common reflecting problems potentially associated with high growth rates and heavier body weights. Large variation existed between pens for all conditions with the prevalence of health conditions in certain pens/farms posing concerns for pig welfare. These preliminary results show that changes in the health conditions recorded reflected the different challenges pigs face at each production stage.

Disclosure of Interest: None Declared

Keywords: Health assessment, Prevalence, Welfare

Herd Health Management and Economy

PO-PT2-131

Survey of Optimal Degradation Agents for Hydrolysis of Swine Cadavers

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Introduction: Many infectious diseases have emerged or re-emerged during the past 50 years in Republic of Korea. There were four outbreaks of foot and mouth disease (FMD) in Republic of Korea. Over 3.45 million animals were slaughtered (33.3% of the existing pigs, 8.4% of dairy cows and 3.4% of cattle, between January 2010 and March 2011). The recycle of swine cadavers due to the animal disease have been problematic in livestock sectors. Hydrolysis method is recently candidate for animal cadavers recycle. In the study, the optimal degradation agents' effect for swine cadaver hydrolysis was analyzed.
Materials and Methods: To select optimal degradation agents of swine cadavers, degradation rates and fertilizer components of pig cadavers were investigated using hydrogen chloride (HCl), potassium hydroxide (KOH) and sodium hydroxide (NaOH) hydrolysis methods. Degradation rates of pig cadavers using HCl, KOH and NaOH were 81.1, 82.8 and 91.6%, respectively.
Results: Total nitrogen (T-N) concentration in degradation solution of pig cadavers using KOH hydrolysis method was higher than that in NaOH and HCl hydrolysis methods. Total phosphorus (P2O5) concentrations in degradation solution of pig cadavers in all hydrolysis methods ranged 0.14 ~ 0.28%. Total potassium (K2O) concentration for KOH hydrolysis method was higher than that for other hydrolysis methods. The concentration of T-N and K2O in degradation solution of pig cadavers by KOH hydrolysis method were higher than that in NaOH and HCl hydrolysis methods.
Conclusion: Thus, to recycle animal cadavers in agriculture, the optimal degradation agent for hydrolysis was KOH.

Disclosure of Interest: None Declared

Keywords: Infectious diseases, Degradation, Swine cadavers, Hydrolysis methods

Herd Health Management and Economy

PO-PT2-132

OVERVIEW OF PIG PRODUCTION IN IBADAN, OYO STATE

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Introduction: Nigeria has long been faced with the problem of inadequate consumption of animal protein. Pig production has therefore been identified as one of the fastest ways of increasing animal protein since they excel other red meat animals, such as cattle, sheep and goats in converting feed to meat. Overview of pig production in Ibadan, Oyo state was carried out, to know the major challenges and different type of management practice among pig farmers.
Materials and Methods: This study was carried out in Ibadan, the largest metropolitan area, which comprises of rural and urban settlements. Six (6) local Governments areas (LGA) were selected and rural communities within these LGAs were used as the study area. In order to assess the overview of pig production in rural and urban communities in Ibadan, a structured self-administered questionnaire were administered to 57 pig farmers in six (6) local government areas in Ibadan. A structured questionnaire was self administered in a face-to-face interview approach to respondent. The respondents were pig farmers (farm owner or managers) or arm attendants. The questionnaire was designed into three (3) different sections, which include: farm details, farmer owner's information and questions on different type of management practice on the farm. The data were analyzed using descriptive statistics.
Results: 86 percent of the pig farms encountered, did not keep farm record and 35 percent of the pig farms had first degree or more. 69 percent of the pig farms prepared feed for their pigs themselves and scavenging is the main source of feed for those practicing extensive type of management. 79 percent of the pig farmers practice intensive type of management and 70 percent of the pig farmers lost their pigs during the rainy season due to diseases, pilfering or insufficient feeding. Taps and wells are the major sources of water for those practicing extensive and semi-intensive type of management, while stream for those practicing extensive type of management. 67 percent of the pig farmers call Veterinary attention if there is disease occurrence on the pig farms and 82 percent of the pig farmers perform self-drug administration of pigs. The common construction materials encountered during this research was concrete blocks, planks and bamboo, and roofing materials vary from zinc and asbestos.
Conclusion: It is noted that pig production was considered a very lucrative business but it is still at a low level of production at the moment. Pig production can be improved upon by adequate record keeping, good extension services and access to veterinarians. Common problems encountered by pig farmers in Ibadan should be adequately addressed so as to enhance pig production in Ibadan.

Disclosure of Interest: None Declared

Keywords: Challenges, Management Practices, Swine Husbandry

Poster Abstracts

Herd Health Management and Economy

PO-PT2-134

The job characteristics leaders need on Central-European swine farms with special regard to management skills

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Introduction: Beyond the technical knowledge and strong work ethic the leaders' good management skills are also required to run a swine farm financially successful. Besides the farm managers the vets also have managerial tasks in setting up an efficient farm operational system. Inadequate management skills can result in inefficient and unprofitable production in the swine herds. In this study we surveyed the job characteristics the leaders would need on the pig farms in Central-Europe with special regard to management skills.
Materials and Methods: From February to May 2014 101 farm leaders, 63 vets and 38 farm managers were personally questioned about the ideal job characteristics including the needful managerial skills on a swine farm by using a questionnaire. The 101 swine farm experts were from 75 farrow-to-finish swine farms with 82,550 sows altogether. 63 farms (66,500 sows) located in Hungary, 11 (14,050) in the Czech Republic and 1 (2,000) in Slovakia, respectively. In the survey out of 31 characteristics the vets and the farm managers had to select the 10 most and the 10 least important job characteristics the leaders need on a Central-European swine farm.
Results: According the 101 respondents the 10 most important job characteristics swine farm leaders need are as follows: technical knowledge (80% of the respondents), quality orientation (77%), accuracy, punctuality (76%), feedback (72%), cooperation (66%), teamwork (66%), strategic thinking (61%), creativity (59%), financial security (56%), and sound mind and body (52%). The 10 least important leader's job characteristics on Central-European swine farms are: spirituality (100%), autonomy (95%), love (85%), empathy (80%), friendship (80%), individual work (66%), loyalty (61%), belief (59%), value orientation (56%) and self-knowledge (52%).
Conclusion: The results show that the technical knowledge and the creative, qualitative team-working are the most preferred managerial characteristics for the swine farm leaders in Central-Europe. Furthermore, the strategic thinking and financial security are also considered to be very important job characteristics. The result orientation, lifelong learning, propensity to innovation, risk-taking and competition are not in the top 10 characteristics. Surprisingly, amongst the 10 least important managerial characteristics the self-knowledge, value orientation and loyalty can be found. These findings impose that the farm managers primarily seek for stability, hence, the Central-European swine farms often respond slowly to the changes in the market environment. This paper highlights the need for management trainings for both farm managers and vets.

Disclosure of Interest: None Declared

Keywords: job characteristics, swine farm management, leaders, skills

Herd Health Management and Economy

PO-PT2-135

An examination of the effect of divergence in feed efficiency on the intestinal immune response following an ex-vivo lipopolysaccharide challenge

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Introduction: Selecting more efficient pigs is a key goal in the future sustainability of the pig industry. Selection for high genetic merit animals has reduced feed intake and body fat reserves with increased growth and feed efficiency and was generally believed to have a negative impact on the immune response. However, the response to infectious diseases and particularly the intestinal innate immune response of pigs with increased feed efficiency has not been fully elucidated. In this study residual feed intake (RFI) was the measure of feed efficiency used as RFI can evaluate differences between animals that are unrelated to production and maintenance requirements. Hence, the objective of this study was to examine select bacterial populations and the gene expression profiles of a range of targets relating to gut health and immunity in the intestine of pigs phenotypically divergent in feed efficiency in: a) the basal state; and (b) following an *ex-vivo* lipopolysaccharide (LPS) challenge of ileal and colonic tissue.
Materials and Methods: Male pigs (BW 22.4 kg (SD = 2.03)) were fed a standard finishing diet for the final 43 days prior to slaughter to evaluate feed intake and growth for the purpose of calculating RFI. RFI was computed for each animal and was assumed to represent the residuals from a multiple regression model regressing average daily feed intake (ADFI) on average daily gain (ADG) and mid-test metabolic body weight (MBW). On day 115, 16 animals (average weight 85 kg, SEM 2.8 kg), designated high RFI (HRFI, inefficient) and low RFI (LRFI, efficient) pigs were slaughtered and digesta from the caecum were collected for microbial population analysis. Similarly, two sections from the ileum and colon were collected to anlyse the gene expression of targets involved in the intestinal immune response using an ex-vivo LPS challenge.
Results: The LRFI pigs had increased *lactobacillus* spp. in the caecum compared to HRFI pigs ($P < 0.05$). RFI groups did not differ in the expression of the measured genes involved in the innate immune system in the basal ileal or colonic tissues ($P > 0.10$). Interestingly, there was an interaction between RFI and LPS for the cytokines *IL-8*, *IL-1*, *IL-6*, *TNF-α*, Interferon-γ (*IFN-γ*) and suppressor of cytokine signaling (*SOCS3)*, with the LRFI group having consistently lower gene expression of these in the colon following the LPS challenge, compared to the HRFI group.
Conclusion: The lower gene expression of SOCS and cytokines following an *ex vivo* LPS challenge supports a theory that a possible energy saving mechanism exists in the intestinal innate immune response to an immune challenge in more feed efficient pigs.

Disclosure of Interest: None Declared

Keywords: feed efficiency , immunology , Microbiota

Herd Health Management and Economy

PO-PT2-136

'Piglet Monitoring' in Northern Belgium: a tool for veterinarians and farmers to control PRRSv and PCV2

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSv) and Porcine circovirus type 2 (PCV2) are recognised as 2 pathogens with a significant economic impact in the domestic pig. There is no official program for the control of both viruses in Belgium. A voluntary project, called 'Piglet Monitoring', offers veterinarians a monitoring tool to provide insights in the herd's infection dynamics of PRRSv and PCV2. This must help the vet to set up a farm specific control program. The pilot started in February 2015 with financial support of the Belgian Fund for Animal Health.
Materials and Methods: Twice a year, 30 blood samples are taken by the herd veterinarian: 10 samples from pigs approximately 4, 8 and 12 weeks (W) of age, resp. The vet also runs a survey on the herd about management and technical parameters. Blood samples are analysed for the presence of PRRSv and PCV2 by PCR, as well as for antibodies against both viruses by ELISA. Analysis of PRRSv is obligatory in the program, testing for PCV2 is optional. When PRRSv is detected by PCR, sequencing is performed. Results and infection trend charts are reported to the vet. The veterinarians are stimulated to use this report for setting up a written control plan that can be delivered to the farmer.
Results: Up till 31st of December 2015, 105 farms are registered for the Piglet Monitoring, of which 81 for both PRRSv and PCV2 analyses and 24 only for PRRSv. Already 22 herds monitored twice, 63 did the first screening. Preliminary results from the first screening on 85 herds are shown: 79% of the herds had a positive PCR PRRSv (28% at 4W, 54% at 8W, 66% at 12W), on 95% of the herds antibodies against PRRSv were detected. Sixty-six herds also tested for PCV2, 48% of these herds had a positive PCR (3% at 4W, 21% at 8W, 47% at 12W) and on all of the herds antibodies against PCV2 were detected. PRRSv sequencing demonstrated that the detected genotype 1 field isolates presented identities ranging from 76% to 94% versus the European vaccine strains whereas the detected genotype 2 strains are identical (96%_99%) to the American vaccine strain. (More details in: 'Piglet Monitoring' in Northern Belgium: phylogenetic and geographic distribution of PRRSv isolates.)
Conclusion: In less than a year, 6% of the target group participated in the Piglet Monitoring program. Almost 80% of the herds tested positive for the presence of PRRSv and half of the herds for PCV2. Antibodies against both viruses were detected on almost all herds. Sequencing revealed that only genotype 1 field strain is present on Flemish herds. Although veterinarians receive results and infection trend charts, this report is not yet sufficiently used to set up a written control plan on the farm.

Disclosure of Interest: None Declared

Keywords: monitoring, PCV2, PRRSv

Herd Health Management and Economy

PO-PT2-138

The influence of the duration of the occupation period of farrowing pens on the economic efficiency of the weaner production

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Introduction: Annual production volumes at 11 Croatian piggeries in 2014 were influenced by the number of farrowing pens 27% and by the index of economic efficiency per pen 73%, depending on the sows' ability 35% and on the efficacy of the use of farrowing pens 38%. The measure of economic efficiency was the number of weaners per pen a year. The numbers of litters (shifts) per farrowing pen a year indicated the efficacy of the use of farrowing pens. Dividing 365 by the number of shifts per farrowing pen a year it could be found out how many days the occupation period of farrowing pen lasted a shift. After that became possible to analyze how the economic efficiency of the weaner production per pen was influenced by the duration of the occupation period of farrowing pen a shift. Suchlike period is determined basically by the production design but it can be changed during the performance at the constructed piggery.
Materials and Methods: At 11 piggeries over Croatia in 2014 the numbers of weaners per farrowing pen a year varied from 82.66 to 140.71 (av. 101), the numbers of shifts per farrowing pen a year from 7.185 to 11.925 (av. 8.659) and the numbers of days of the occupation period of farrowing pen a shift from 30.61 to 50.80 (av. 43.24). The relationship between the duration of the occupation period of farrowing pens and the index of economic efficiency per pen was studied performing the calculations as it had been taught in Ames, Iowa, by G. W. Snedecor and W. G. Cochran.
Results: The coefficient of correlation between the duration of the occupation period of farrowing pens a shift (X) and the index of economic efficiency per pen (Y) was $r_{X/Y}$ = -0.908, the determination coefficient $r_{X/Y}^2$ = 0.8245. The increase of the duration of the occupation period of farrowing pen a shift from 30.61 days (X) resulted with the decrease of the number of weaners per farrowing pen a year (Y) according to the regression equation Y_C = (-10.737) – 2.294X, the decrease from 50.80 days resulted with the increase of the index of economic efficiency in accordance with the equation Y_C = 1.002 – 2.294X.
Conclusion: It was estimated in 2007 that 140.9 weaners per pen a year would be probable with 11.58 weaners/litter and the duration of the occupation period of farrowing pens 30 days a shift. At the farm ŽITO in Lipovača, Croatia, during 2014 the farrowing pens were occupied per shift 30.61 days on average and 140.71 weaners were weaned with the mean of 11.799 weaners/litter. At 11 piggeries over Croatia the economic efficiency of the weaner production per pen was determined 82.45% by the duration of the occupation period of farrowing pens a shift.

Disclosure of Interest: None Declared

Keywords: farrowing pens, use

Poster Abstracts

Herd Health Management and Economy

PO-PT2-140

Hematological parameters in stillborn and pre-colostral liveborn piglets

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Introduction: A high number of stillborn piglets represent both an ethical and economic challenge in the pig production. Asphyxia in piglets has been identified as a major cause of stillbirth and is related to the oxygen and blood supply of the piglet. Oxygen carrying ability of blood is related to hematological parameters like hemoglobin, red blood cells and mean corpuscular hemoglobin concentration. Therefore, the objective of this study was to determine if differences exist in hematological parameters between stillborn piglets and liveborn piglets before colostrum intake.
Materials and Methods: A total of 39 sows from two farrowing batches in a Danish farrow-to-finish herd were studied at the time of farrowing. Altogether 20 liveborn piglets and 43 stillborn piglets from these sows were included. Dead piglets collected around farrowing were autopsied to determine whether they were stillborn. All fully developed piglets with uninflated lungs were considered as stillborn. From each sow, two stillborn and two liveborn piglets (maximum) were selected at convenience. Blood was withdrawn from the stillborn piglets directly from the heart and vena cavae during autopsy while it was withdrawn from the superior vena cava in case of liveborn piglets. The liveborn piglets were sampled before colostrum intake. The EDTA stabilized blood was subjected to complete hematology testing. Student's t-test was used to compare the mean hematological parameters in stillborn and pre-colostral liveborn piglets.
Results: Hemoglobin in stillborn piglets was significantly lower (100.81±24.04 g/l) g/l) than in pre-colostral liveborn piglets (115.42±7.84 (p=0.0007). Similarly, red blood cells (p<0.0001), hematocrit (p=0.008), hemoglobin distribution width (p<0.0001), absolute reticulocytes (p<0.0001), percentage of reticulocytes (p<0.0001), and reticulocyte mean cell hemoglobin concentration (p<0.0001) was significantly lower in stillborn piglets compared to pre-colostral liveborn piglets.
Conclusion: Stillborn piglets had lower hemoglobin values than pre-colostral liveborn piglets. Hematological parameters may be associated to survival of the piglets during parturition, and high levels of hemoglobin and oxygen carrying capacity of blood should therefore be aimed for. However, the changes in hematological parameters in stillborn piglets might also be due to changes in blood cells after death over time. Further studies are required to study the extent of these changes.

Disclosure of Interest: None Declared

Keywords: Hematology, Liveborn, Stillborn

Herd Health Management and Economy

PO-PT2-142

Collaborative approach for endemic disease (PRRS) control in the province of Quebec, Canada

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Introduction: Controlling and containing endemic and emerging diseases is known to require a collaborative work between all producers sharing the same territory. In the province of Quebec, PRRS virus infection has been an endemic disease affecting the production sector for the last 25 years (1990-2015). It is known to cause losses of CAN$40 million per year. Developing collective and collaborative skills for PRRS control is therefore an excellent model to develop collaborative disease control methodologies in general.
Materials and Methods: Collaborative PRRS monitoring and control procedures have been implemented in the province of Quebec in a 3-phase process over 4 years: 1) Pilot projects (2011-2012); 2) Optimization and adaptation process (2012-2014) and 3) Implementation (2015). The 2 first phases (2011-2014) were carried out on about 200 farms and 5 zones and were largely financed through public money. Since 2015, all swine production units in Quebec have been encouraged to join the provincial monitoring program and local group initiative for the control of PRRS.
Results: At the end of 2015, 40% (1121/2780 sites) of the production sites in Quebec have subscribed to the PRRS monitoring program and registration is on-going. This represents more than 180 producers, 27 veterinarians and different partners like laboratories, the Quebec swine producer association and the Quebec Centre for Swine Development (CDPQ). The CDPQ has developed a database system and diverse tools for data collection and information delivery. The 5 pilot zones are pursuing collaborative actions to control the PRRS and some producers and veterinarians from other zones are in the implementation phase of local and regional PRRS control strategies. The collaborative work experience acquired through the PRRS control strategies (endemic disease) has provided a beneficial building block for the Quebec swine sector's efficient reaction to the arrival of the PED (emerging disease). Implementing a collaborative endemic disease control strategy on a larger scale remains complicated since it requires sharing of sensitive swine health related data and information and it requires private financial investment from producers to support the collaborative services.
Conclusion: Although the importance of collaborative work for the control of many diseases is recognized in the scientific community, implementing this on a large scale remains a major challenge. A disease having major economic impacts and affecting numerous farms within a territory provides an opportunity to develop necessary collaborative skills for controlling swine disease. The experience acquired with endemic disease management can then be used efficiently when any emerging disease arises.

Disclosure of Interest: None Declared

Keywords: Collaborative approach, Endemic, PRRS control

Herd Health Management and Economy

PO-PT2-143

Effects of antibiotic reduction in the Netherlands on the sales mix of a swine practice

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Introduction: In the Netherlands in 2005-2006 the discussion about prudent use of antibiotics was intensified. In 2010-2011 new legislation in The Netherlands forced pig farms and the veterinarians to further reduce the use of antibiotics. Dutch veterinary clinics are allowed to sell pharmaceutical products to clients and the sales of drugs and vaccines significantly contributes to the income of veterinary clinics. This article shows how the sales mix of pharmaceutical products, sold for use at pig farms, has changed over the last 10 years by categorizing them as Preventive use or Curative use.
Materials and Methods: De Oosthof veterinary clinic is a top 5 player in the Dutch swine market, together with 10 swine veterinarians we give veterinary support to about 300 pig farms. Our practice management system (Viva 3.0, Corilus) allows us to mark a product as Preventive or Curative. Visits, consultancy, vaccines, iron, monitoring, blood samples and reports are marked Preventive. Antibiotics, painkillers and anthelmintics are marked Curative. From 2006 until 2015 (December excluded) we collected data on the sales mix of all the sow farms in our practice.
Results: In 2006 of our gross sales 43%was considered Curative and 57% Preventive. This Curative/ Preventive sales mix was more or less stable until 2009, when the discussion on prudent use of antibiotics started. From 2009 until 2013 the sales mix changed. In 2014 and 2015 the sales mix is stable again: in 2015 (December excluded) 89% of our gross sales was Preventive.
In 2006 vaccines were 67% of the Preventive sales. In 2015 vaccines were 91% of the Preventive sales. PCV2 and Mycoplasma vaccines being number 1 and 2 in the vaccine sales. Costs charged for consultancy time were the same over the years; 7% of the gross sales.
Conclusion: In the Netherlands the total health costs of sows, including their piglets until 25 kg live weight, from 2005 to 2012 increased from 56 € to 75 € per sow per year. In comparison, over the same period the costs of antibiotics decreased from 19 € to 8.5 € per sow per year.
The sales mix of our veterinary clinic is in line with this: it has strongly changed towards more sales of products for Preventive use. Consultancy sales are relative equal in all the years, but as the total health care costs are rising, the absolute sales for consultancy time increases. Sales of vaccines are a significant part of our veterinary clinic profit. Sales of antibiotics become less important year after year. We consider the use of Preventive products important as a part of sustainable pig production.

Disclosure of Interest: None Declared

Keywords: antibiotic use, economic

Herd Health Management and Economy

PO-PT2-146

Long and short term evolutions in the prevalence of pneumonia in slaughter pigs in three EU countries between 2011 and 2013.

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Introduction: Respiratory diseases play a major role in the pig industry. The scoring of the post mortem lesions can give a good indication of the general health status of a pig herd. It can be used as a tool to monitor health in pig herds at a national level. The aims of this study were: 1. to assess the prevalence of lesions in fattening pigs for the years 2011-2013 in different EU countries. 2. to study the evolution over time (per year and per season) of different lesions in slaughter pigs.
Materials and Methods: Existing datasets were provided by three European countries (A-C). Slaughterhouse data were collected by the countries in different ways. Data were obtained from integrators or governmental organizations. In every country, data were obtained from fattening pig herds. Evolutions in the prevalence of pneumonia were studied per year and per month. Prevalences of pneumonia in slaughtered pigs were given in country A and B; in country C the prevalence of pneumonia referred to the percentage of pigs which were condemned due to pneumonia.
Results: In country A data from 696 herds was obtained, in country B data of 407 herds was used and in country C data from 1630 herds were obtained. The overall prevalences of pneumonia were: 35.0% (A), 27.90% (B) and 1.50% (C). The yearly prevalence for the years 2011, 2012 and 2013 were, respectively: 31.6%, 35.3%, 36.5% (A), 30.2%, 27.4%, 26.3% (B), and 1.44%, 1.64%, 1.42% (C). In country A, the increase was significant, whereas in country B and C the differences per year were not significant. In country A, pneumonia tended to decline in winter (Nov, Dec, and Feb) and increase in summer (Aug, Jul), but this was not significant. In country B, a significantly higher prevalence of pneumonia was also seen in July (33.4%). In country C, a seasonal effect could not be studied due to the nature of the dataset.
Conclusion: The different dataset-providers used different methods to obtain their data. As a consequence, it was not possible to make a comparison between countries. Pneumonia prevalence was high throughout the years in countries A and B. In country C, the prevalences are lower, but differences in registering of the data did not allow the comparison of prevalence between countries. No consistent trends could be seen over the years. A higher prevalence of pneumonia was seen during the autumn and winter months and July.
This work was conducted under the EU-funded PROHEALTH project.

Disclosure of Interest: None Declared

Keywords: None

Poster Abstracts

Herd Health Management and Economy

PO-PT2-147

Disease incidence in Swedish pigs

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Introduction: Antimicrobial usage is comparably low in Swedish pig production, but previous studies have shown that there is room for improvement in some herds especially for suckling piglets. The aim of this study was to investigate for which diseases treatments were most commonly performed in different age groups and what antimicrobials were used.
Materials and Methods: Treatment records for 3 batches were collected from 9 pig herds, which included 1,095 sows, 15,346 suckling piglets, 12,570 weaning piglets and 9,919 fattening pigs. Treatments including antimicrobial substance and total volume were recorded by disease symptoms and age group.
Results: The most common cause of treatment for suckling piglets was arthritis (58.1% of treatments), followed by diarrhea (30.5%), for which 17.2 and 9.1% of the piglets were treated, respectively. In weaners, diarrhea was most common (67.7% of treatments) followed by arthritis (15.0%). However, in total, only 4.9 and 1.1% of the weaners were treated for these symptoms. Arthritis was the dominating cause of treatment in fatteners, (58.3%) for which 1.6% were treated. For lactating sows, MMA was most common (50.8% of treatments), followed by mastitis (40.6% of treatments), corresponding to 22.1 and 17.6% of sows treated for these symptoms. Trimethoprim sulphonamide (TS) (40.4%), amoxicillin (28.4%) and neomycin (22.6%), were used for treatment of diarrhea in suckling piglets. For treatments of arthritis, benzylpenicillin (PC) dominated (86.4% of treatments). Diarrhea in weaning pigs was treated with colistin (46.0%), TS (25.8%) and tylosin (12.9%). PC was mainly used for treating arthritis in fatteners (78.4%) whereas TS (62.5%) or tiamulin (37.5%) was used for diarrhea. In sows, MMA was treated with TS (74.2%) or PC (25.8%) and mastitis with PC (99%).
Conclusion: In the studied population of pigs, treatments were common only for suckling piglets and lactating sows, where arthritis and diarrhea, and MMA and mastitis, were the most common causes for antimicrobial treatments. To reduce antimicrobial use and improve health in these herds, special attention should be paid to disease preventive measures during the farrowing and suckling period. The frequent use of colistin to treat diarrhea in weaners needs to be addressed in particular due to the recent finding of plasmid-mediated polymyxin resistance.

Disclosure of Interest: None Declared

Keywords: None

Herd Health Management and Economy

PO-PT2-154

THE USE OF AN AEROSOL DESINFECTANT IN WEANING AND FINISHERS BUILDINGS IMPROVE PRODUCTION PERFORMANCE

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Introduction: Introduction: Viral infections (PRRS & SIV) in pigs predispose secondary bacterial infections and promote the use of antibiotics in feed or water. Respiratory infections are disseminated by aerosols within the building increasing the infectious pressure . All in all out systems separate different batches by washing and disinfection process between them but many farms in Mexico do not manage an strictly system and pigs decrease it´s performance between 5 and 10 weeks age. (1,2) **Objective:** Apply by aerosol a safe disinfectant to pigs in weaning and growing stages and analize it´s effect over air total bacterial count and batch performance in each one.
Materials and Methods: Material and Methods: Nine 1,100 weekly production batches piglets were divided in two groups (550 each one) housed in different buildings from 21 to 70 days age and 70 to 161 days age. Treatment groups were fogged with a Triclosan 2% desinfectant in a 1:100 dilution during 49 consecutive days in weaners and 78 days in growers. Daily fogging times were 3 & 8 minutes in weaners and growers respectively with a 0.5µ particle size using a comercial nebulizer. Control groups were fogged with water only. Each building were monitored in 5 specific sites for air total bacterial count using a biocolector (Biomerieux Lyon France) in 7 days intervals, total air sampled volumen was 250 liters. Bacterial count was performed in Petri dishes impacted by the collected air. Performance parameters in each batch were; mortality, average daily weight gain and Feed-efficiency. Data were analized by a T-Sudent test using a SPSS V.15.0 software.
Results: Results. There was a reduction in total bacterial count in each building, weaners showed an increase in total counts (Forming Colony Units) from 15.0 to 35.0 x 10^3 FCU/250 lts/air from 3 to 7 weeks age, treated barns showed 5.0 to 15.0 in the same period. In finishers, negative controls were from 25.0 to 35.0 x 10^3 in a 12 weeks period and treated from 20.0 to 5.0 10^3 FCU´s. Productive performance, measured as ADWG and Feed Efficiency was; weaners 0.485 vs 0.496 and 1.58 vs 1.45 (controls vs treated respectively) in finishers 0.831vs 0.863 and 2.61 vs 2.35 with statistical differences.
Conclusion: Discusion and conclusions. There are several reports about the bacterial endotoxin effect over pig growth, that requires high energy levels to maintain an immune response, reduction in this challenge will improve growth,. This alternative offer a possibility to maintain low infectious pressure with reduction in antibiotic use (3). **Ref.**1) Dee S, et al.. Can J Vet Res. 2005; 69:58–63. 2) Dee S. et al Can J Vet Res 2005; 69:64-70. 3) Gonyou HW et al. Dis Swine, 9th Ed. Blackwell Publishing, pp. 1027-1038.

Disclosure of Interest: None Declared

Keywords: Aerosol, Disinfectants, Triclosan

Herd Health Management and Economy

PO-PT2-155

Brucellin BM; a new skin test allergen for resolving the problem of False Positive Serological Reactions (FPSR) in swine brucellosis diagnostic tests

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Introduction: The brucellae are gram-negative bacteria causing brucellosis, an infectious disease affecting a variety of mammals including domestic ruminants and swine. *Brucella suis* biovar 2 is presently restricted to continental Europe, where it represents an emerging problem causing abortions, infertility and a high economic impact in pig farms. Despite this, the EU is currently considered as officially free from swine brucellosis, and surveillance based solely on serological tests performed only for trade and as a routine surveillance in semen production.
Materials and Methods: Current serological tests detect antibodies to the O-polysaccharide (O-PS) moiety of the *Brucella* smooth lipopolysaccharide (S-LPS), the dominant surface molecule present in all smooth brucellae. These O-PS tests include the Rose Bengal (RBT), Serum Agglutination (SAT), Complement Fixation (CFT), Fluorescence Polarization (FPA) and indirect or competitive Enzyme-linked Immunosorbent Assays (iELISA and cELISA, respectively). Despite being officially recommended for the diagnosis of swine brucellosis but these tests lack specificity to discriminate brucellosis from the FPSR caused by bacteria sharing O-PS epitopes with *Brucella* S-LPS, such as *Yersinia enterocolitica* O:9. A skin test with classical brucellin (Brucellergene OCB) is currently used for discriminating brucellosis from FPSR. However, this allergen is obtained from *B. melitensis* B115 strain, which possesses intracytoplasmic O-PS, which can be a source of important diagnostic problems.
Results: Aquilón CYL, in cooperation with two international reference groups on *Brucella* research (CITA and University of Navarra), has developed a new skin test allergen obtained from an O-PS free natural rough mutant of *Brucella abortus*, which has been named Brucellin BM. Considered as MUMS (Minor Use/Minor Species) by the EMA (European Medicines Agency), the company is currently evaluating the safety and efficacy of the Brucellin BM skin test with the objective of being registered as soon as possible by the centralised procedure.
Conclusion: Brucellin BM would become an international reference test to solve the diagnostic problems caused by the FPSR in swine and other domestic species to avoid the current trade problems within the European Union, including unnecessary culling.

Disclosure of Interest: None Declared

Keywords: Brucella, skin test, swine

Herd Health Management and Economy

PO-PT2-160

Development of a tool to study the spatial-temporal pattern of swine pathogens in a highly dense pig population area.

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Introduction: Spatial and temporal analysis has become an integral component of animal health studies, with increasing sophisticated methods being applied to understand the epidemiology of pig diseases. The knowledge of this epidemiology could be critical to develop disease control programs at regional level. The goal of the present work was to develop a practical tool to collect information to study the spatio-temporal pattern of pig diseases in a highly pig dense pig population area with the final goal to develop unique control programs for pig diseases at regional level.
Materials and Methods: Between 40 and 50 pig production companies from Spain were included in this study from 2012 to 2015. These companies were operating in farrow-to-finish, two-site and three-site production systems and included a total of 209.000 sows and 2.520.000 pigs. The veterinarian responsible for each farm sent information about each clinical case which includes: Date, type of farm, age of affected animals, clinical and lesions observed, severity of the clinical case and presumptive and confirmed diagnosis. The information was filled using an application (App) for mobile phone (GSP App in google play and apple store) to make easier this procedure under field conditions. The collected data each month were represented in charts. A statistics descriptive was calculated for each presumptive and confirmed diagnosis during the period 2012 to 2015 using the month as experimental unit.
Results: The most prevalent disease at regional level was post-weaning diarrhea associated to overgrowth of *Escherichia coli*. However, it is more prevalent the presence of respiratory than digestive diseases during the rearing period. Moreover, it is close the number of clinical cases by month due to swine influenza virus, Porcine Respiratory and Reproductive Syndrome virus, *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae*, *Pasteurella multocida* and porcine circovirus type 2. On the other hand, Salmonelosis, exudative epidermitis and atrophic rhinitis are the diseases with the lowest prevalence at regional level.
It is not possible to describe a temporal pattern for many pig diseases with the exception of *Actinobacillus pleuropneumoniae* and *Mycoplasma hyopneumoniae* where it is observed a significant increase of clinical cases during the winter period. Until now, there is no enough data to describe precisely spatio-temporal patterns for many pig diseases.
Conclusion: The analysis of the spatio-temporal patterns of pig diseases is a necessary tool to develop control programs at regional level. An App could be a practical tool to gather this information under field conditions.

Disclosure of Interest: None Declared

Keywords: pig diseases, Spatial, Temporal

Poster Abstracts

Herd Health Management and Economy

PO-PT2-171

Investigating in-transit infections of breeding stock

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Introduction: Transport of breeding stock for genetic dissemination occurs at local, regional and even international levels. Most of this movement of animals occurs on trucks travelling through different swine dense regions which can expose pigs to local pathogens. However, data on in-transport infections is scarce. The objective of this study was to describe the breeding stock transport events between USA/Canada and Mexico as a means for understanding in-transit risk of infection.
Materials and Methods: Records of exported boars and gilts originating from PIC's nucleus herds in Canada or the United States into Mexico during the last 5 years were considered for this study. A subset of these records was then extracted comprising breeding stock animals that were imported into Mexico's PIC boar studs or multiplication units. Records included date of export, herd of origin, number of pigs and destination herd.
Results: A total of 33,383 pigs (7,949 boars and 25,434 gilts) were imported into Mexico between July 1st 2011 and December 15th 2015 through a total of 435 shipments. Out of these, 62 shipments were strictly for PIC units comprising in total 6,703 (4,801 boars and 1,902 gilts) pigs. On average, 12.4 shipments occurred per year ranging between 8 and 15.
Shipments of pigs occurred throughout the year with 53% and 47% of them occurring between the months of October-March and April-September, respectively.
Pigs originated from a total of 8 herds. These herds were located in the Canadian provinces of SK and QC or in the United States (KS, OH, SD, MN or MO). Approximately, pigs travelled between 1,000 and 4,300 kilometers in order to reach the Mexican border. There were 9 recipient herds (7 boar studs and 2 farrow-to-wean) in this study located in the Mexican states of CHIH, JAL, PUE, QRO, SON and YUC. Depending on their port of entry to Mexico, pigs traveled between 250 and 1,300 kilometers between the Mexican border and the recipient herd quarantine unit.
The nine recipient herds in this study continue to remain free of major swine pathogens (PRRS, PEDv, PDCoV, Mycoplasma hyopneumoniae) after continuing to receive breeding stock animals from Canada or the United States.
Conclusion: This study sheds light into the understanding of the risk of in-transit infections of breeding stock of animals that travel hundreds of kilometers to their final destination.
Our data suggests that the risk of in-transit infections is low when transport biosecurity measures are in place together with planning a route where trucks avoid driving through pig dense regions. More studies are required to fully understand the risk of in-transit infections.

Disclosure of Interest: C. Corzo Conflict with: PIC Employee, L. Castrejon Conflict with: PIC Employee

Keywords: Transport

Herd Health Management and Economy

PO-PT2-172

Factors associated with high mortality in finishers

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Introduction: High mortality in the pig industry is a welfare problem as well as it reduces the farmers income. The Danish Pig farmers have decided to reduce the mortality with 20 % before 2020 compared to the 2011 level. For each percent the mortality is reduced in the finisher barns, the gross margin per produced finisher pig is increased by 0.70 euro. The objective of this study was to provide knowledge of differences in management, health, feeding- and housing conditions that impact on mortality in the finisher barn.
Materials and Methods: The hypothesis was that identification of differences in management, health, feeding- and housing conditions in herds with high and low mortality can identify risk factors for high mortality rates in finisher barns.
The study was performed as a case-control study in finisher barns with high or low mortality. Finisher units with low mortality had a mortality ≤ 2.6%, and finisher units with high mortality had a mortality ≥ 4.2%. Each farm was visited by a veterinarian and a comprehensive questionnaire was filled in during the herd visit.
The initial statistical univariate analysis was performed by logistic regression (Proc Logistic in SAS). Factors significant at P < 5% were considered for further analysis. Factors were selected by two criteria. There should be a plausible biological link to mortality and it should be possible to change the factor. To check for confounders, factors that were biologically plausible but hard to change were also included in the multivariate analysis. The results are presented as Odds Ratios (OR) for being a finisher barn with high mortality. An OR > 1 indicates an increased risk and OR < 1 indicates a reduced risk.
Results: A total of 43 finisher barns, where 21 had high mortality and 22 had low mortality, were included in the study. The multivariate analysis showed that herds with PRRS type 2 had an Odds Ratio (OR) of 32.5 for being a finisher herd with high mortality compared to a herd without PRRS type2. Herds with poor internal biosecurity, where pigs were moved to other barns, had an OR 14.7 for having high mortality. For each hour the duration of quarantine time for persons was increased, the OR was 0.75.
Conclusion: This study indicates that the risk of being a finisher barn with high mortality is associated with disease (PRRS), internal biosecurity (moving pigs between barns) and external biosecurity (hours of quarantine).

Disclosure of Interest: None Declared

Keywords: Case control, Finisher mortality, Risk factors

Herd Health Management and Economy

PO-PT2-173

Economic valuation of different farrowing management protocols in hyperprolific sows systems

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Introduction: Pork production has experienced tremendous genetic progress, which has been achieved an increase in litter size and survival of young piglets; this resulting in an increase in the production of piglets per sow per year which get the highest possible return. All this leads to the continuous search for suitable methods of handling this type of sucker by veterinarians and pig producers. The aim of this study is to analyze different kinds of pig management in the maternity ward of the same pig farm and calculate its economic value to an age close to sacrifice in order to know what gives us better economic results
Materials and Methods: The productive results (average weight, average daily gain and mortality of piglets at weaning and at the end of the nursery and fattening phase) the usual handling of the piglets on the farm (control group) with three different management groups are compared piglets group "level out " (piglets are moved to the nursing mother), group "holes in farrowing place" (farrowing sows are moved to the piglets being adopted) and group "milk supplement" (artificial milk is given piglets with a special installation). We have also noted the daily intake of sows during lactation and recorded for the low mortality in each operation.
Results: The results indicate that, productively, the handling control and "holes in farrowing place" have superior results at weaning and at the end of the transition (Control = 7,178Kg and 18,202Kg; "gaps in maternity" = 7,131Kg and 17,360Kg) without however piglets management "milk supplement" experience significant exponential growth observed in cebo1 phase where exceed average weight (54,194Kg) ½ GMD (756,928g) to other handling (P = 0.05). There are differences in costs for each operation, the most economic management " level out" (100.5 €) versus management "gaps in maternity" (€ 103.4) which is more expensive.
Conclusion: 1. The final cost for handling " level out" is lower than the rest.2. productively best results correspond to the handling and monitoring "gaps in maternity" while litter productive results show similar or in some cases lower.3. The management "milk supplement" shows much better results in weight fattenig1 and GMD fattening1, so it would be interesting to apply animal low birth weight

Disclosure of Interest: None Declared

Keywords: farrowing management, Hyperprolific, reduce cost

Herd Health Management and Economy

PO-PT2-174

Comparative study on risk factors and management of PRDC from the Central European vets' and farm managers' point of view

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Introduction: In a successful Porcine Respiratory Disease Complex (PRDC) management the close cooperation of farm managers and vets are essential on the swine farms. That is why it would be very important that the farm managers consider the risk factors, the risk assessment, the control and supervision of PRDC more or less on the same lines with the vets. The aim of this study was to compare the opinion of vets with that of farm managers on the effective PRDC management.
Materials and Methods: From February to May 2014 39 farm managers (32 from Hungary, 6 from Czech, 1 from Slovakia) and 37 vets (26 from Hungary, 10 from Czech, 1 from Slovakia) working on the same farrow-to-finish swine farms were personally questioned about their opinion on the risk factors and management of PRDC applied in their herds by using the ResPig™ (MSD AH) questionnaire. The surveyed farm parameters were evaluated on a 0-to-4 point scale, where 0 = excellent, 4 = need of urgent intervention. The results were compared by the major PRDC factors, which are based on the related farm parameters, and on the severity of PRDC pathogens, and by the contributors (V/FM; V = average vet points, FM = average farm manager points).
Results: The average scores of the different PRDC factors are as follows: environment (farm isolation, herd health site security, quarantine, AIAO, hygiene level by itself) 0.83/0.59; farm management (owner's attitude, staff, feed quality, feeding system, water, veterinary service, data management) 0.11/0.03; housing (climate, stocking density, separation of sick animals) 1.43/0.57; production parameters (disposal, ADG, FCR, uniformity, animal health costs) 1.43/0.95; lung health (clinical signs, pathology, epidemiology) 0.98/0.70; other diseases 2/1; slaughterhouse lung-scoring 0.96/0.96. The average scores of the severity of PRDC pathogens are: PRRS 1.33/1.22; M. hyo 1.26/1.3; APP 1.38/1.1; SIV 1.23/1.10; HPS 1.08/0.91; AR 0.84/no data; PCV-2 1.35/1.2.
Conclusion: The vets considered most of the PRDC factors (environment, farm management, housing, production parameters, lung health and other diseases) to be more considerable than the farm managers did, albeit we would have expected the opposite. At the same time the evaluations of the severity of PRDC pathogens on the surveyed farms showed much more similarity, yet the atrophic rhinitis was not identified by the farm managers at all. Based on the results it can be concluded that the farm managers in Central-Europe should more often conduct a systematic farm audit to avoid the operational blindness and to better understand the underlying factors of PRDC.

Disclosure of Interest: None Declared

Keywords: PRDC – risk factors – vets – farm managers

Poster Abstracts

Herd Health Management and Economy

PO-PT2-175

Economic Analysis of a PRRS Resistant Dam Line: go for Resistance or Performance?

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Introduction: With recurring PRRS outbreaks, producers are wondering if disease resistant dam lines could be more profitable. Three dam lines were tested in a commercial farm in Quebec (Canada). The objective was to see how they would perform during an outbreak of PRRS.
Materials and Methods: Gilts from three different genetic dam lines were introduced in an empty 650-head commercial sow barn. To standardize the rearing period, gilts were raised together after nursery (25 kg). All gilts were PRRSv naïve; proximity (< 150 m) to an infected farm insured contamination, which occurred before the first parity.
For the first three parities, performance and veterinary treatments were recorded on an individual basis. Diagnostic tests were also carried out to follow the evolution of the herd health status. Data covered 601 sows (203 for line A, 198 for line B and 200 for line C).
Mortality rates and veterinary treatments were collected for 12,922 piglets and 8,536 pigs. Average daily gain (ADG) and feed conversion ratios (FCR) were collected for six lots of pigs.
The economic analysis looked at the profitability of the sow and finishing units, using average prices for feed, pigs and piglets in 2015 in Quebec, as well as actual prices for the treatments used during the project. For the finishing unit, a sensitivity analysis was performed with various pig and feed prices.
Results: Line A and C proved statistically the most prolific ($P \leq 0.05$), with an average of 11.07 piglets/weaned/parity and 10.29 piglets for line B. Line A also proved to be more resistant to disease requiring less ($P \leq 0.05$) treatments, both with antibiotics (56 % less treated sows) and anti-inflammatories (44% less). Line A had the highest margin (income over feed + treatment) per sow ($685), with C a close second ($683) and B in third place ($607).
During the finishing phase, line A proved more disease resistant with a lower ($P \leq 0.05$) mortality rate (4.2% vs 8.3% for the other two). But line A suffers from lower ($P \leq 0.05$) growth performance, especially compared to line C. When mortality is controlled, line C is more profitable than A (+$5.0) with lower feed and fixed costs. For a disease outbreak with a high mortality rate, line A is more profitable (+$4.7), but sensitivity analysis shows it holds true only when feed prices are low or pig prices are high.
Conclusion: The optimal choice between a dam line that is more resistant to disease and another that offers superior growth performance will vary from one farm to another. Resistance does not translate necessarily into better profits. Many factors need to be taken into account, like expected pig and feed prices, but also the disease risk threatening the farm and its magnitude (high/low mortality).

Disclosure of Interest: None Declared

Keywords: disease resistance, PRRSv

Herd Health Management and Economy

PO-PT2-177

Cross-fostering practices: a survey of North American sow farms

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Introduction: Cross-fostering (CF) is a common practice throughout the swine industry. The proper CF practices have created ongoing research and discussion around its relevance, plus to what extent CF should be utilized. In order to determine the relevance and extent of CF occurring in the North American swine industry, survey questions were written and disseminated to several veterinarians representing multiple farms and companies.
Materials and Methods: A series of 46 questions were written and edited by the authors. FluidSurveys™ software was utilized to put the questions into a digital user-friendly format. The survey was sent by email and attached via a hyperlink. The survey was split into sections to capture the size and scope of the farm, health status, sow management, gilt management, piglet processing, cross-fostering and split-suckling protocols. CF was defined as any disturbance in the natural born litter. Although confidentiality was assured to each participating farm, information such as herd veterinarian and farm name were requested to insure authenticity and negate duplicity.
Results: A total of 56 responses were tallied and analyzed. These responses represented just under 160,000 sows with an average herd size of 2,800. The number of hours per day that farrowing room workers were available ranged from 4-24 with an average of 12.8 and a median of 10. The number of pigs weaned/sow/year ranged from 18.8-32.5 with an average of 26.85. Pre-weaning mortality ranged from 6.6-22% with an average of 12.83 and a median of 12. Farms with PRRSV scores of 2a-4 were included in analysis; this excluded 2 farms that were unstable. The other health concerns reported that hindered cross-fostering included: *Rotavirus,* Influenza, bacterial scours and *Mycoplasma hyopneumoniae*. CF occurred on 98% of farms. The majority of farms did CF in less than 25% of litters, and most CF had ceased by 7 days of age. Only 4% of farms had significant (>25% of litters) CF after 7 days. Over half of the farms created runt litters, and 80% of those were made in the first 24 hours of life. 74% of farms created starve-out litters. Split-suckling was split with 51% utilizing this practice. The main reason (78%) for CF decision-making was to save more pigs.
Conclusion: The degree of CF in the survey was higher than expected. The majority of farms utilized CF in a minimal manner to create runt and starve-out litters. Comparing the farms that utilized the most CF with those that used the least did not reveal significant differences in pre-weaning mortality or pigs weaned/sow/year. In addition, the best farrowing room performance was associated with more hours of labor devoted.

Disclosure of Interest: None Declared

Keywords: Cross fostering, pre-weaning mortality, PSY

Herd Health Management and Economy

PO-PT2-180

INFORMATION CAMPAIGN IN THE CONTEXT OF A REGIONAL SWINE DYSENTERY ERADICATION PROGRAMME

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Introduction: *Brachyspira* (*B.*) *hyodysenteriae* is the causative agent of swine dysentery (SD). Rodents are considered to be one of the most important vectors within and between herds, especially in regions with several infected herds. To prevent re-infection of herds during and after a regional eradication programme, a coordinated and area-wide rodent control in all farms and in all feed- and food-storing facilities is assumed being indispensable.
The aim of the present study is to develop an information campaign that will accompany a regional eradication programme. The intention is the appropriate information of all involved persons and their compliance in respect to the eradication per se and to the necessity of rodent control.
Materials and Methods: The target region comprises 3 confirmed *B. hyodysenteriae* positive pig herds and 9 other pig herds. Moreover, 13 farms housing livestock other than pigs, 8 vets, 20 food related operations, 3 feed operations and 1 sewerage treatment plant were identified in the target region and being important for the project.
The information campaign will be based on identification of A) key persons, of B) key components and C) targets, and D) the preparation of strategic approaches. Besides oral communications, printed information material like flyers, information brochures, posters, and informative articles for the local press will be designed and distributed.
The 12 pig owners were individually informed during an on-farm consultation (A). The pig health service, the major pig traders and the veterinarian of 8 of the 12 pig farms were informed in a kick-off meeting (B & C). Other stakeholders were individually informed. The livestock owners in the region, their veterinarians, owners of food and feed operations, and other applicable persons will be informed during a meeting before the project starts (D). Topic of this information event will be, amongst others, the professional rodent control which has to be performed ideally by a specialised rodent control company.
Results: All 12 pig owners accepted in participating in the SD eradication programme. Overall reaction to the proposed project was positive. Especially owners of the negative herds accepted easily. Individual owners were reluctant in the beginning due to expected high costs and extra amount of labour but could be convinced when pointing out the benefits of SD free pig farming. The stakeholders assured support of the eradication programme.
The preparatory information process exceeded 1 year.
Conclusion: Although this time span could have been tightened in some points, we assume that a sufficiently long lasting period is necessary in order to perform a sustainable information campaign.

Disclosure of Interest: None Declared

Keywords: Brachyspira hyodysenteriae, Communication, Rodent control

Herd Health Management and Economy

PO-PT2-181

Biosecurity practices in swine herds in Poland

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Introduction: The biosecurity is defined as the implementation of measures that reduce the risk of disease agents (viral, bacterial, fungal or parasitic) being introduced and spread within the pig herds. It is documented that higher level of biosecurity improved animal welfare and production efficiency, and lead to a reduction of antimicrobials use. Proper biosecurity also may help prevent and/or cut the spread of exotic diseases among domestic pigs at the country level. The aim of the study was to determine biosecurity level in swine herds in Poland especially in terms of African Swine Fever outbreaks in Poland.
Materials and Methods: A cross-sectional survey was conducted in 377 Polish pig herds between Oct 2013 and Feb 2015. The fulfillment of questionnaire contained 45 questions was carry out by personal interviews on farms by 56 veterinarians. Answer 'yes' to the each of twenty-nine questions concerning biosecurity measures was scored as 1 point. Sum of scores determines overall herd biosecurity score. Associations between herd's biosecurity score and each of selected factors (location of the herd, pig density in the farm area, herd size, herd type, age of owner, age of veterinarian) was done by Kruskal-Wallis test followed by Dunn's test for multiple comparison. Significance was taken as $p<0.05$.
Results: The percentage 'yes' respondents for some selected questions was as following: fence around the farm (57.8%), disinfection baths at the entry of the farm (28.4%), use of boots and clothes provided by the farm (48.3%), serological/PCR examination of purchased animals (21.0%), consumption of pork is not allowed in the farm (16.7%), cleaning and disinfection of buildings (81.7%), cleaning and disinfection of vehicles (26.8%), all-in-all-out (57.3%). The biosecurity score was associated with herd location. The herds from North part of Poland had the best mean biosecurity score (16.0) compared to other locations ($p<0.05$). Farrow-to-wean farms had highest biosecurity score (18.2), then wean-to-finish (12.5) and do worse results had farrow-to-finish (10.9) ($p<0.05$). Moreover, the large herds had higher biosecurity level than medium and small size herds ($p<0.05$).
Conclusion: Results clearly indicate that appropriate biosecurity and management practices in Polish pig herds are generally unsatisfied. The biosecurity level in majority of pig holdings may be insufficient to prevent the transmission of highly contagious diseases. In addition, the biosecurity level was related to location, type and size of a herd.

Disclosure of Interest: None Declared

Keywords: biosecurity, management, pigs

Poster Abstracts

Herd Health Management and Economy

PO-PT2-188

Evolution of medication costs over a 10 years period from National technical-economic database on French Pig farms

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Introduction: The control of the medication costs is a major challenge in pig industry to optimize production costs and meets the national plan of reduction of the antibiotic use in veterinary medicine. Medication costs are collected in the National technical-economic database (GTE). This is an indirect annual monitoring of antibiotic use from a large network of farms national distribution. In this paper, the evolution of medication costs over the last 10 years (2004-2014) in French Pig farms is analysed.
Materials and Methods: Medication costs are analysed from results collected in GTE database in two types of herds: farrow-to-finish herds (n> 1475 farms) and fattening herds (n> 339 farms). Several medication costs were considered: total costs, preventive costs with 2 sub-categories, vaccines and livestock management products, curative cost with the orally-administered medication and antibiotic and anti-inflammatory injections.
Results: In farrow-to-finish herds, the total medication costs decreased significantly by 0.76 €/100 kg carcass (-12%) between 2004 and 2014, in relation to the decrease in orally-administered medication (- 0.65 €/100 kg carcass, -40%), in antibiotic and anti-inflammatory injections (- 0.47 €/100kg carcass, -42%) and also in livestock management products (- 0.26 €/100 kg per carcass, -18%). During the same period vaccination, costs increased (+ 0.28 €/100 kg carcass, +11%). Over those 10 years, the levels of curative medication decreased by 41% and were lower than those of preventive medication (- 2.27 €/100 kg carcass in 2014).
Medication costs for fattening herds also decreased significantly by 0.72 €/100 kg carcass (-24%) between 2004 and 2014, in relation to the decrease of orally-administered medication (- 0.65 €/100kg carcass, -42%) and antibiotic and anti-inflammatory injections (- 0.33 €/100 kg carcass, -63%). However, livestock management products and vaccines remained stable for this category of farms. Over the 10 years, the level of curative medication decreased by 47%. In 2014 the preventive medication costs were higher than those of curative medication (+ 0.11 €/100 kg carcass).
Conclusion: The decrease in medication costs associated with a decreased use of curative treatments and an increased use of vaccines, meets the expectations of Human and Animal Health Authorities and society. This is due to improvement in the health status of farms in connection with the development of vaccinations and biosecurity practices contributing to the success of the actions to reduce antibiotic use.

Disclosure of Interest: None Declared

Keywords: antibiotic use, medication cost, vaccination

Herd Health Management and Economy

PO-PT2-189

HAEMATOLOGICAL VALUES IN SOWS FROM FIVE DIFFERENT ONE-SITE PIG FARMS IN SLOVENIA

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Introduction: Haematological examinations of blood are commonly used in veterinary medicine, but they are rarely performed for routine evaluation of health status and welfare in pigs. The main reasons are difficulty in collecting blood, low value of individual animals and the fact that pigs are rarely treated individually. The aim of this study was to evaluate haematological variables as additional tool for monitoring herd health and nutrition in sows from five small one-site farms previously included in a serological study of selected pathogens.
Materials and Methods: The study was carried out from January until September 2014, on five one-site farrow to finish Slovenian pig farms and involved 272 breeding sows. All farms were free of Aujeszky's disease and Classical swine fever as well as porcine reproductive and respiratory syndrome (PRRS) which was successfully eliminated. Sows were dewormed regularly. Blood samples were collected from anterior vena cava. Haematological analyses were performed on the day of sampling with an automated haematological analyser Scil Vet abc Plus (Horiba). Haematological variables; red blood cell count (RBC), white blood cell count (WBC), haematocrit (Ht), haemoglobin concentration (Hb), erythrocyte indices (MCV, MCH and MCHC) and platelet count (PLT) were measured. Differential white blood cell counts were determined according to standard procedures (smears stained with Hemacolor (Merck)). Statistical analysis of haematological data was performed using the SPSS 22.0. Descriptive statistics and analysis of variance were calculated.
Results: Mean values of haematological variables in sows were RBC 5.63±0.75 $x10^{12}$/L, Hb 11.59±1.28 g/dL, Ht 34.94±4.38 %, MCV 62.26±3.29 fL, MCH 20.65±1.40 pg, MCHC 33.25±1.07 g/dL, WBC 15.21±3.04 $x10^{9}$/L and PLT 274.31±74.81 $x10^{9}$/L. In differential white blood cell count mean percentages were 48.48±12.62 % for segmented neutrophils, 6.82±3.85 % for eosinophils, 0.21±0.51 % for basophils, 43.11±11.48 % for lymphocytes, 1.34±1.44 % for monocytes and 0.03±0.17 % for band neutrophils. The farm influenced statistically significant all investigated variables ($P<0.05$) except band neutrophils. In one herd lower mean values of RBC, Hb and Ht were found and in two herds higher haematological values were found in comparison to other investigated herds.
Conclusion: The differences in haematological values found between farms could be attributed to the herd health status and partially to differences in nutrition between farms. We think that haematological examination can be useful for detection of subclinical diseases in a herd.

Disclosure of Interest: None Declared

Keywords: haematology, herd health, pigs

Herd Health Management and Economy

PO-PT2-191

An EcoHealth Baseline Survey of Smallholder Swine Production in the Municipality of San Simon, Pampanga, Philippines

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Introduction: Swine production is extremely important in the Philippines, with eighty percent of swine raised by smallholder farmers (SH). In 2008, a severe epidemic of Porcine Reproductive and Respiratory Syndrome decimated the swine population in the Municipality of San Simon, 50 kilometres north of Manila. Since then, many SH have decided not to continue swine production. We have commenced a four year project in this area. The project will follow the principles of EcoHealth research: systems thinking, transdisciplinary research, participation, sustainability, equity and knowledge to action. As a first step, we conducted a baseline survey in the 14 Barangays (villages) in this Municipality to better understand the distribution of active, compared to inactive, SH and identify the major constraints affecting swine production.
Materials and Methods: The survey was conducted from June to August 2015. The target population was active SH (currently raising swine/raised swine within the last month) and inactive SH (had functional pens and facilities for raising swine but had not raised swine for the last month). The sample size was as close to a census as possible with active and inactive SH identified using house-to-house methods. The survey teams consisted of a trained enumerator and a Barangay representative. GPS co-ordinates were used to plot the location of farmers and descriptive statistics were used to summarise other data.
Results: One thousand and eighty two SH were interviewed for the survey. Of these, 629 were active (58%) and 453 inactive (42%). The ratio of active to inactive SH varied between Barangays. During the survey period, the swine population of the Municipality comprised 593 sows, 97 gilts, 28 boars, 1365 piglets, 1758 fatteners (wean to finish).The greatest constraint identified by SH was finance. Others included lack of space and disease. From a disease perspective, SH reported that diarrhoea was the most common problem in piglets, fatteners and sows. Poor appetite and lethargy in sows was also reports as important.
Conclusion: There is a potential for increased pig production even if sow numbers remain unchanged. A good performing 600 sow commercial herd should be producing 10,500 slaughter age pigs in a 12 month period. Most SH are keen to participate in the next steps of the project. This will entail; (i) focus groups and semi-structured interviews to better understand the smallholder system and associated constraints and to estimate enterprise budgets and (ii) farm visits by a veterinarian to gather data on productivity, observed health and management.

Disclosure of Interest: None Declared

Keywords: Ecohealth, Production, Smallholder

Herd Health Management and Economy

PO-PT2-192

Biocheck.ugent®: a quantitative tool to measure biosecurity at pig farms

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Introduction: Herd biosecurity is the combination of all measures taken to reduce the risk of disease introduction (external) and spread (internal), aiming to ensure health and productivity. Ensuring good biosecurity is of crucial importance in modern pig production and no protocol is suitable for every herd. To balance biosecurity and management and to quantify a herd-specific biosecurity status, Biocheck.ugent® was developed. The tool enables an accurate and objective quantification of this status and allows for comparison between herds and in time.
Materials and Methods: The Biocheck.ugent® scoring system is a risk-based weighted scoring system for an objective evaluation of the biosecurity status of pig herds and was developed after several years of scientific research. It is relevant to practice and continuously updated. Data is collected via the Biocheck.ugent® questionnaire. It is subdivided in external and internal biosecurity, with 6 subcategories per part and 2-13 questions per subcategory. It is available for free on www.biocheck.ugent.be in multiple languages. It results in a weighted score (0-100) for internal (IB), external (EB) and overall biosecurity (OB), meaning that a higher score is achieved when better biosecurity is implemented.
Results: Since its launch (12/'08), Biocheck.ugent® for pigs acquired 1207 entries (real data) from 28 countries by 11/'15. The assessment tool has already been used in several scientific studies in Belgium (BE), Sweden (S), France (FR), Germany (DE), Ireland, Denmark to objectively evaluate biosecurity and the associations with factors like health, production characteristics, antimicrobial use. For four countries we have more than 60 observations allowing to provide country specific averages: herds in BE scored in average 59% in OB, 66% in EB and 52% in IB; in FR scored 59% in OB, 61% in EB and 55% in IB; in DE scored 60% OB, 66% EB, 53% IB; in S scored 63% OB, 67% EB, 58% IB. EB is found higher than IB in most cases. Many measures are common in practice, e.g. farm-specific clothing and shoes for visitors (96%). Others should be practiced more often, e.g. verifying efficiency of cleaning and disinfection (2.5%). A positive correlation between EB and IB scores has been identified. Herds repeatedly using the system prove clearly an improvement over time.
Conclusion: Biocheck.ugent® scoring system is a widely applied system allowing for objective and accurate assessment of herd biosecurity. The results indicate that in most cases there is room for improvement of the biosecurity. The tool -freely available for use- can be a valuable support for herd veterinarians in counseling their clients towards better disease prevention.

Disclosure of Interest: None Declared

Keywords: biosecurity, Management Practices, scoring

Poster Abstracts

Herd Health Management and Economy

PO-PT2-193

The volume and economic efficiency of the weaner production depending on the number of sows, their ability and on the efficacy of reproductive method

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Introduction: At any piggery annual production volume can be calculated multiplying the number of sows by the ratio of the number of weaners produced and the number of sows exploited during a year. Customary measure of economic efficiency of the weaner production per sow is the product of the number of weaners per litter and of the number of litters per sow a year. Former factor indicates the sows' ability, the later one is the measure of the efficacy of used reproductive method. The Croatian Agricultural Agency Report 2014 showed that sows (PIC, TOPIGS, Croatian Hybrids etc.) over Croatia were able to have weaners differently and that they were exploited on different ways (concerning reproductive methods E, F, H or I). It was possible to analyze how much the increase of the piglets' production was influenced by the increase of the numbers of sows and how much economic efficiency of the weaner production depended on the sows' ability and on the efficacy of reproductive method.
Materials and Methods: The data collected at 11 Croatian piggeries during 2014 did the materials in this research as the percentages of numbers registered at the least farm SIZIM 1 having 294 sows, 758 litters, 8,428 weaners, 28.67 weaners per sow a year, 2.578 litters per sow a year and 11.119 weaners per litter. At observed piggeries the numbers of sows varied from 100 to 885, the numbers of litters from 100 to 998, the numbers of weaners from 100 to 816, the numbers of weaners per sow a year from 79 to 107, the numbers of litters per sow a year from 83 to 100 and the numbers of weaners per litter from 93 to 115. The sample regression line and the prediction equations, analyzing the multiple regressions, were formed as it had been taught in Ames, Iowa, by G. W. Snedecor and W. G. Cochran.
Results: The regression of the increase of the production volume (Y) on the increase of the number of sows (X) could be expressed by the line derived from the equation $Y_C = 0.963X - 1.808$. Calculating with the number of sows (X_1) and of the number of weaners per sow a year (X_2) the prediction equation of the number of weaners produced during the year was found to be $Y_C = 0.933X_1 + 2.194X_2 - 196.462$. The equation of prediction of weaners per sow a year, calculating with the number of weaners per litter (X_1) and with the number of litters per sow a year (X_2), was $Y_C = 1.078X_1 + 1.462X_2 - 150.561$.
Conclusion: The magnitude of annual production volume at 11 piggeries in Croatia during 2014 was influenced by the number of sows 32%, depending on the index of economic efficiency of the sows' exploitation 68% (on the sows' ability 29%, on the efficacy of the reproductive method 39%).

Disclosure of Interest: None Declared

Keywords: efficiency, piggery, volume

Herd Health Management and Economy

PO-PT2-194

Effect of Corynebacterium lysate (Ultra-corn®) on growth performance under field condition

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Introduction: One of the fundamental biological functions in pork production is growth. If efficient growth is inhibited, the amount of feed needed to produce 1 kg gain is inevitably affected as well. The growth appears to be associated in various ways with immunity. Mounting and maintaining a competent immune system, is thought to be a nutritionally demanding process that requires trade-off decisions among competing nutrient demands for growth. An ultrasonicated lysate of *Corynebacterium* (Ultra-corn®, Virbac) as an immunostimulating compound may activate the cell-mediated immune response when detected by antigen presenting cells which are the first line defense cells of the host. This study was performed to test the effect of Ultra-corn® on overall growth performance such as average daily weight gain (ADG), reduced variable weights within pen and health status by one time administration to nursery piglets at their weaning.
Materials and Methods: A GP farm housing 580-sow herd with a two-site production system was selected. Every week 200 to 250 piglets are weaned and delivered at about 70 days of age to the finishing farm. 120 weaned piglets were selected and assigned to the Ultra-corn® treated group (UC group; n=60) with more variable and lower weight and the untreated control group (CTL group; n=60) with more uniform and heavier weight. The UC group was given a single injection of Ultra-corn® (1 ml per pig) intramuscularly at 4 days post weaning and the CTL group remained untreated. Both groups were maintained without other treatment except minimum vaccination. The live weight of each pig was measured at weaning and before delivery to the finishing unit to analyze the ADG. As a base line data, the number of delivered pigs, weaning age, and delivering age were recorded.
Results: The body weight was 6.9 ± 1.24 kg in the UC group and 7.3 ± 0.67 kg in the CTL group at weaning and 25.5 ± 1.47 kg in the UC group and 24.3 ± 1.59 kg in the CTL group when delivered at 68 days of age. The ADG was about 40 gram higher in the UC group (454.4g) than the CTL group (412.9g). Those results were visually confirmed by the farm's own inspection and also statistically significant.
Conclusion: The generalized response to an infection is to redistribute nutritional and energy resources away from anabolic and maintenance processes to the vitally important metabolic processes driving immunity and disease resistance. In other words, the immune status of low body weight pigs and variable weights within pen could be said not stable. This study suggested that the Ultra-corn® enhanced the growth performance through encouraging overall immune status of growing pigs.

Disclosure of Interest: None Declared

Keywords: ADG, Corynebacterium, Immunity

Herd Health Management and Economy

PO-PT2-205

EFFECT OF TILDIPIROSIN (ZUPREVO® 40mg/ml) AT WEANING ON PRODUCTION DATA IN A FARM WITH RESPIRATORY DISEASE

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Introduction: Tildipirosin is a newly developed macrolide indicated for treatment and metaphylaxis of swine respiratory disease associated with *A. pleuropneumoniae*, *P.multocida, B. bronchiseptica* and *H.parasuis*. All these pathogens are widely prevalent in commercial farms. This study was designed to demonstrate the effect of Zuprevo® 40mg/ml (MSD Animal Health) on production parameters in a farm with chronic presence of respiratory disease in the nursery.
Materials and Methods: The study was carried out in a closed, 250 sow herd located in southern Spain. The farm had historical prevalence of chronic respiratory disease in the nursery. Although clinical signs were not very severe (4% average mortality from 4 to 8 weeks of age), some growth retardation was observed. In order to adjust antibiotic usage to a responsible level, no feed medication was administered during all nursery period. A total of 286 weaned 28 day old piglets were randomly allocated into two treatment Groups: Control (146 piglets located in 7 pens) and Zuprevo (140 piglets located in 7 pens). The Zuprevo Group was treated with 0,7ml of Zuprevo® 40mg/ml via i.m. (4 mg/kg BW), while the Control Group remained untreated. Mortality was recorded during the nursery period (5 weeks). Piglets were weighed as a group, per treatment pen, when entering the nursery and 5 weeks later. Weigh increase and ADWG were recorded and compared considering the pen as the statistical unit (Pearson Chi Square Test, Levene Test and ANOVA).
Results: Animals from the Zuprevo Group had a higher body weight at the end of the nursery period. Body weight gain from 4 to 9 weeks of age was 650g higher in the Z group (C 8,47kg vs Z 9,12kg; p=0,01). Consequently, ADWG was also significantly higher (C 242g/d vs Z 260g/d; p=0,01). An economic analysis based on the treatment cost and body weight at 9 weeks of age, Zuprevo piglets had an extra benefit of 1,27€ per piglet (included cost of treatment). No differences were found between groups in regards to mortality rates (C 4,8% vs Z 5,7%; p=0,9).
Conclusion: Using Zuprevo® (40mg/ml) at weaning in farms with respiratory disease is efficacious and profitable, provides heavier and healthier piglets to the fattening period, and can be considered a good alternative to feed medication in the nursery period.

Disclosure of Interest: None Declared

Keywords: Economics, macrolide, respiratory

Herd Health Management and Economy

PO-PT2-206

Monitoring PRRS and Mycoplasma hyopneumoniae naïve populations with current serologic tests

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Introduction: High health herds monitor their population through active surveillance using diagnostic tests. In certain instances, test results may misclassify individuals due to inaccurate tests (false positive or negatives) generating confusion and perhaps forcing unnecessary and costly interventions. The objective of this study was to understand how tests perform in two PRRS and Mycoplasma hyopneumoniae (Mhyop) naïve herds.
Materials and Methods: Serologic test results from two nucleus herds located in the United States and Canada that are constantly monitored for PRRS and Mhyop were used for this study. Data were summarized and analyzed in an excel spreadsheet based on s/p ratios and proportion of negative, suspect and positive results through the study period. Samples were considered positive or suspects according to manufacturers' recommendations. In addition, data from PCR test results from the same farm were used to confirm that the herd maintained its naïve status for the two mentioned pathogens.
Results: Data between January 2013 and September 2015 were obtained. A total of 21,795 and 5,729 sera were serologically tested for PRRS and Mhyop, respectively. For PRRS, 39 samples yielded an s/p between 0.3 and 0.39 whereas 69 (0.31%) samples yielded an s/p ratio equal or above to 0.4. As for the positive samples, a total of 56 samples yielded an s/p ratio between 0.4 and 0.8 and 13 samples that had an s/p ratio above 0.8 with the maximum being 1.65. For Mhyop, there were 251 samples with an s/p ratio between 0.3 and 0.39. With regards to the samples considered positive by the test, 124 (2.1%) yielded an s/p ratio of equal or above to 0.4. There were 115 samples that yielded an s/p ratio between 0.4 and 0.8 and 9 samples that had an s/p ratio above 0.8 with the maximum being 1.59. The herd continues to maintain the naïve status for both PRRS and Mhyop based not only on the absence of clinical signs but also on the constant PCR negative results.
Conclusion: The results from this study provide insight into the understanding on the accuracy of serologic tests and the probability of obtaining false positive results. The data demonstrated that none of the tests has a perfect specificity and the ELISA for Mhyop tended to generate more suspect and false positive results. When monitoring naïve populations with an imperfect test, the veterinarian should always keep in mind that there will be a probability of obtaining false positive results due to imperfect analytical specificity due to different factors.

Disclosure of Interest: None Declared

Keywords: Diagnostic test, ELISA, Monitoring

Poster Abstracts

Herd Health Management and Economy

PO-PT2-207

Economic value of productivity differences following a change in PCV2 and Mycoplasma hyopneumoniae vaccines using historical production data

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Introduction: The objective of this analysis was to use a retrospective quasi-experimental study design with historical production data, and an enterprise budgeting model to estimate the economic value of differences in key productivity indicator (KPI) outcomes attributed to a change in porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* (*M. hyo*) vaccines in growing pigs following efforts to control porcine reproductive and respiratory syndrome virus (PRRSV).
Materials and Methods: The analysis was performed for an 8,000 sow, 3-site production system in the Midwest USA. The breeding herds were in American Association of Swine Veterinarians (AASV) PRRSV category I (Positive Unstable) prior to the spring of 2013 when efforts to stabilize the herds for PRRSV were initiated. Prior to February of 2014, pigs were vaccinated for PCV2 and *M. hyo* with a single, 2 ml dose of a combination vaccine (Vaccine A) at 3 weeks of age. In February of 2014, in response to higher than expected finishing mortality following the PRRSV control efforts, the vaccine was changed to a 2 dose regimen, 1 ml per dose, of Circumvent® PCV-M G2 (PCVM-G2) (Merck Animal Health, Summit, NJ), administered at 3 and 6 weeks of age. The first treatment group included groups of finishing pigs started from 2011 to 2013 and vaccinated with Vaccine A. The second included groups of pigs started in 2014 and vaccinated with PCVM-G2. Average daily gain (ADG), feed conversion (FCR), mortality rate and cull rate were analyzed as response variables in linear regression models. Explanatory variables included treatment group (TrtGrp), weight of pigs at placement in the finisher and days on feed as potential confounding variables. An enterprise budgeting model in the ResPig® Management System was used to evaluate the economic value of KPI differences between the treatment groups. The least square means of each KPI from the regression analysis were used in the economic simulator.
Results: For 226 groups of finishing pigs, 177 with Vaccine A and 49 with PCVM-G2, differences between Vaccine A and PCVM-G2 were significant at $p<0.05$ for ADG (762 g/d, 844 g/d; $p<0.001$); FCR (2.98, 2.79; $p<0.001$) and mortality (8.06%, 5.39%; $p=0.001$) but not for cull rate (4.8%, 4.9%; $p=0.087$). The estimated value of the improved KPIs after the switch to PCVM-G2 was $14.96 per pig marketed. The largest contribution to the increase was $5.54 per pig marketed from the 82 g/d increase in the least squares means estimate of ADG.
Conclusion: The approach used addresses the challenge of isolating changes in KPI attributed to the vaccine change from those attributed to other causes like efforts to control PRRSV.

Disclosure of Interest: D. Holtkamp: None Declared, B. Thacker Conflict with: Employee of Merck Animal Health, J. Creel Conflict with: Employee of Merck Animal Health

Keywords: Economic benefit, Mycoplasma hyopneumoniae, porcine circovirus type 2

Herd Health Management and Economy

PO-PT2-210

PROPHYLACTIC USE OF ANTIMICROBIALS IN NEWBORN PIGLETS AND THEIR EFFECTS ON PERFORMANCE OF SUCKLING PIGLETS

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Introduction: Aiming to increase pig productivity, commercial swine farms have opted not to use the all-in all-out management, what has led to an increase in infection pressure instead, prophylactic and metaphylactic use of antimicrobials has been a commonly used in farms in Brazil to prevent enteric infections, arthritis and encephalitis. However, indiscriminate use of antimicrobials can disturb intestinal homeostasis, rendering piglets prone to diseases as a result of changes in intestinal microbiota. Studies evaluating the segregated early weaning medicated found that in spite of better performance and health status of animals in the long term, there was a higher incidence of septicemia outbreaks. These outbreaks may occur as a result of the absence of colonization by pathogens during the period when the piglets are still protected by antibodies acquired from the colostrum. Thus, the aim of this study was to evaluate the prophylactic use of ceftiofur in neonatal piglets on performance and frequency of days with normal feces during the suckling period.
Materials and Methods: Shortly after birth, all piglets received the same care, such as airway clearance, cutting and disinfection of the umbilical cord and breastfeeding of colostrum. On the first day of life, piglets were homogenized and assigned into two treatments: ceftiofur (CEF, 5 mg/kg, n=89) and control (CTR, buffered saline, n=85), both administered in a single dose via the IM route. The piglets were weighed on the 1st, 7th, 14th and 21st days of age and average daily gain during periods (1-7, 7-14 and 14-21days of age) was evaluated. The fecal score was evaluated daily and classified as: 1 (normal), 2 (soft) and 3 (diarrhea). Data were analysed using the PROC MIXED of SAS (2002), according to block design containing treatments as main factor. Statistical significance for all tests was set at $p<0.05$.
Results: There was an interaction ($p<0.05$) between treatment and period since piglets of the CEF treatment were 6.5% heavier than the CTR at 21 days of age. The same interaction ($p<0.05$) was observed for piglets of the CEF group regarding average daily gain; this variable was 12,79 and 10.60% higher than CTR in the periods 7-14 and 14-21 days of age, respectively. No interaction was observed between treatment and period for frequency of days with normal feces neither for the isolated factors.
Conclusion: Prophylactic use of ceftiofur improved performance of piglets. However, no difference in the frequency of days with normal feces was seen.
Acknowledgement: FAPESP Process nº 2014/27182-4

Disclosure of Interest: None Declared

Keywords: newborn piglets, prophylactic antimicrobials

Herd Health Management and Economy

PO-PT2-222

Implications for finishing house efficiency of growth rate variability from weaning to sale weight

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Introduction: Variability of post-weaning growth can result in either an increase in the sale of lighter weight pigs or an extended finishing period. This poses management challenges as to what is the optimal strategy for de-stocking finishing buildings in order to produce the optimal financial return per finishing pig place.
Materials and Methods: An 1800-finisher pig place straw-bedded, naturally ventilated (ACNV) house managed on an all in-all out basis comprising 20 pens was used to house pens of 90 gilts or boars on a split-sex basis. Pigs entered at approximately 53kg and were sold at 112kg live weight. Pigs were fed ad libitum; 2 sample pens were weight monitored at weaning, on entry, and at weekly intervals for the last 8 weeks of the finishing period.
Results: Weaning weight at 24-28 days of age had a mean weight of 7.39kg (3.08-12.7kg) SD 1.6kg and 22% coefficient of variation. At entry to finishing at 104 days of age and a mean weight of 54.4kg (29.5-86.5kg) SD 8.1Kg CV 14.9%. First sales draw (5.5% of pigs) was at 139 days of age when the overall house mean pig weight was 91.6kg (58.5-122.5kg) SD 10.1kg, CV 11.0%.

At draw 4, 68% of pigs had been sold at a mean live weight of 111.7kg; the residual 562 pigs (32%) then had a mean live weight of 99kg. These pigs were sold in 4 further successive weekly draws of 17%, 11%, 3 and 1.5% of the total pigs with mean live weights of 111.6, 110.9, 108.4 and 106.2kg respectively. If all of the pigs had been sold at draw 4 then the house could have been re-stocked 4 weeks sooner thus improving housing efficiency but with the penalty of reduced full-value pigs being sold. Use of a spreadsheet incorporating all production costs and sales revenues enabled the most efficient return / pig place to be determined. If all pigs were sold at or before draw 2 a reduced margin of -£4.79 pig place / year occurred due to not optimising sale weight. Selling all pigs prior to, or at draw 5, an additional margin of £13544 / year, £2597 / batch (£1.48 / pig) or £7.52 per pig place per annum could be achieved. This being the most profitable outcome.
Conclusion: Variability of pig weight and growth performance is an inevitable part of pig production. Efficient pig production requires such variability to be managed in order to maximise housing efficiency and margin / pig place / annum. Knowledge of variation and variable factors such as costs of production and market prices enables the optimal number of weekly sales draws to be identified. The optimal number of sales draws is not fixed and will vary in accordance with relative changes in the above factors.

Disclosure of Interest: J. Richardson Conflict with: Consultant

Keywords: Efficiency, profitability, Variability

Herd Health Management and Economy

PO-PT2-223

The volume and economic efficiency of the weaner production depending on the number of farrowing pens, on their use and on the sows' ability

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Introduction: The construction of the indoor piggery could be paid out, if 103 piglets would be weaned per farrowing pen a year – it was estimated by American Service Specialists in 1978, when 8 weaners per litter could be planned and less than 9 piglets were weaned per litter at large pig units in Croatia and elsewhere. At the 63rd EAAP Annual Meeting in Bratislava, Slovakia, 2012 the average of 11.62 weaners per litter was reported to be expected in the herd of hybrid hyperprolific sows. At the same time 151.5 weaners per farrowing pen a year were estimated to be achieved applying the methods of The Safe Commercial Swine Husbandry. The Croatian Agricultural Agency Report 2014 did possible to analyze how much the number of weaners produced in the year was influenced by the number of farrowing pens at the piggery and how much annual production volume depended on the efficacy of the use of farrowing pens and on the indicator of the sows' ability.
Materials and Methods: The data collected at 11 Croatian piggeries during 2014 were the materials in this research taken as the percentages of numbers registered at the least farm SIZIM 1 having 84 farrowing pens, 758 litters, 8,428 weaners, 100.3 weaners per farrowing pen a year, 9.024 litters (shifts) per farrowing pen a year and 11.119 weaners per litter. At observed piggeries the numbers of farrowing pens varied from 100 to 929, the numbers of litters from 100 to 998, the numbers of weaners from 100 to 816, the numbers of weaners per farrowing pen a year from 86 to 140, the numbers of shifts per farrowing pen a year from 80 to 122 and the numbers of weaners per litter from 93 to 115. The statistics were calculated as it had been taught in Ames, Iowa, by G. W. Snedecor and W. G. Cochran using the scientific calculator CASIO fx-82ES.
Results: The regression of the number of weaners produced in the year (Y) on the number of farrowing pens at the piggery (X) could be expressed by the line derived from the equation $Y_C = 46.500 + 0.832X$. Calculating with the number of farrowing pens (X_1) and with the number of weaners per pen a year (X_2), the prediction equation of annual production volume was $Y_C = 0.599X_1 + 1.632X_2 – 21.70$. The prediction equation of the index of economic efficiency per pen was $Y_C = 0.942X_1 + 0.875X_2 – 81.740$, where the number of shifts per farrowing pen a year was X_1 and number of weaners per litter was X_2.
Conclusion: At 11 Croatian piggeries in 2014 annual production volume was influenced by the number of pens 27% and by the index of economic efficiency per pen 73%, depending on the sows' ability 35% and on the efficacy of the use of farrowing pens 38%.

Disclosure of Interest: None Declared

Keywords: efficacy, pens, piggery

Poster Abstracts

Herd Health Management and Economy

PO-PT2-224

The regional PRRS control model developed in Taiwan

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Introduction: PRRS (Porcine Reproductive and respiratory disease) is one of the major highly infectious disease globally, and it makes huge negative impact on breeding herd by abortion storm, increasing weaken piglets, decreasing birth weight, and increasing stillborn on reproductive performance. In the past years, Taiwan swine industry gradually change the production from continuous flow to batch system, and we found the herd healthy status improving with the achievement of All-in, all-out and production stability. Taiwan is an isolated island in the subtropical climate region. Based on the geographical advantage and positive result of production system improving, Taiwan provide the suitable conditions to developed the disease control and eradication model for pan-asia area as a option. The purpose of this project is to develop the PRRS control and eradicated model by batch production system introducing with immune stabilized protocol.
Materials and Methods: The project operated on the highly restricted Vally terrain region in the east south side of Taiwan. Totally have 7 farms participate in the trial, and all of participators are operating the farrow-to-finish unit in the one site system, and the scale from 200 sows to 500 sows. Before starting the trial, the PRRS infectious surveillance collected from all participating farms based on RT-PCR and ELISA. Whole trial have three stages, the purpose of the first stage is to stabilized farm production and adapt to all-in all-out program with pig flow design, and environment investigation. The second part is to immune stabilized by vaccination program. The third stage of trial is to operate the control program by partial partial depopulation or depopulation depend on the farm PRRS status.
Results: Currently, the all participators are acclimatized to the three week batch production system, and build up the production monitor system, finished the stage one status. Additionally, the PRRS status supervising result showed the majority of participators keep PRRS positive stabilized status, and the other farms improve from unstable status to stable status.
Conclusion: According to the trial performance so far, stage one of controlling model is effective to stabilize farm production, and it also show the positive impact of mortality of nursery pigs, and reproductive performance, but the trial still need to prove the PRRS status improving of farm after the stage two trial to immune stabilized by vaccine and suitable management strategy to block the internal circulation route and enhance the gilt acclimation program.

Disclosure of Interest: None Declared

Keywords: Batch management system, PRRS control, Regional disease control program

Herd Health Management and Economy

PO-PT2-225

Assessment of the efficiency of waterlines cleaning protocols in post-weaning rooms

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Introduction: To guarantee the best quality of water from the source to the animal troughs, it's important to be aware that water quality can be adversely affected by the formation of biofilms in distribution systems, which represent persistent reservoir for potentially pathogenic bacteria. In addition, the presence of biofilm in water distribution systems makes disinfection difficult or it can decrease the efficacy of oral treatments administered to the animals like vaccines, antibiotics or nutritional factors. Cleaning measures to eliminate the biofilm or to limit its development are part of health management in farms.
For many criteria regarding water quality, poultry producers are more aware than pig farmers. The differences in their practices concern the monitoring of water consumption and the maintenance of water pipes. In pig husbandry, weaning is a critical management period since piglets become exposed to social, environmental as well as nutritional changes which might be regarded as stressful events. Digestive disorders are the main health problem and could be linked with unadapt water quality. In this study, we have chosen this sensitive period to evaluate in pig farms the effects of different mechanical and chemical waterlines cleaning protocols, similar to those used in poultry farms.
Materials and Methods: Two different protocols similar to those used in poultry farms have been tested. They combined (i) the mechanical action of draining, (ii) one detergent (either an alkaline or an enzymatic one), (iii) another draining state and finally (iv) one acid used at an antibacterial concentration. The experiment has been set up during the down period in post-weaning rooms. To follow the bacteriological quality of water during protocols, we have counted the total flora at 22°C and 37°C in water. Before and after the experiment, cotton swabs were applied into the pipes to evaluate biofilm.
Results: Bacterial concentration in water increased along the pipelines: total flora was higher at watering place than at the entry of the building. Both protocols combining mechanical and chemical procedures reduced total flora, improved water quality and cleanliness of pipes.
Conclusion: Our results show that waterlines cleaning protocols (transposed from those used in poultry farms) reduce water's total flora and could be part of the health prevention measures for troubles which are linked to a poor water quality. The improvement of water management could be also used to reduce antibiotic consumption during this period. It would be interesting to measure the re-contamination of water flowing in pipes in order to adapt protocols mixing optimization of water quality for animals and convenience for farmers.

Disclosure of Interest: None Declared

Keywords: pig, water management, water quality

Herd Health Management and Economy

PO-PT2-226

Lung and pleura lesions in Swiss slaughter pigs before and after implementation of a national eradication program against EP and APP.

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Introduction: Respiratory diseases represent one of the most common problems in modern pig farms in the world and cause significant economic losses due to poor daily weight gain, reduced feed efficiency and increased treatment costs. *Mycoplasma (M.) hyopneumoniae* as the cause of EP and *Actinobacillus pleuropneumoniae* (APP) are together with PRRSV and Influenza the most common causes of respiratory diseases in pigs. Dealing with infected or subclinical infected pigs and air borne transmission of pathogens are main risk factors for the spread of respiratory diseases. Hence, eradication of EP or APP on a single farm is not sustainable. In 1996 – 2004 a national wide program was carried out in Switzerland to eradicate EP and APP. All farms suffering from APP identified by the authorities had to carry out a complete depopulation of all pigs. Farms with EP were obligated per law to perform either a complete or a partial depopulation-program of pigs younger than 10 months of age in the period of August 15 to September 1. Because of the closed market, Switzerland is still free of PRRSV.
Materials and Methods: Five years after the end of the eradication program, 24,276 slaughtered pigs from 639 farms were investigated in four different abattoirs to evaluate lung and pleura lesions as well as lesions of carcasses and organs. These data were compared with a former study, conducted before the eradication program. Lungs with bronchopneumonia or lesions suggesting APP were investigated for *M. hyopneumoniae* and APP by PCR and culture, respectively.
Results: Of the examined pigs, 91.2% of the lungs, 94.4% of the hearts and 95.5% of the livers showed no macroscopically visible lesions. Compared to the study conducted before the start of the eradication program, the numbers of lungs without lesions increased from 56 to 91.2%, lungs with bronchopneumonia decreased from 21 to 3.6%, lung lesions caused by APP from 2 to 0.5% and generalized pleurisy from 21% to 5.2%. *M. hyopneumoniae* was not detectable by PCR in any of the 85 lungs with bronchopneumonia. Aspiration pneumonia followed by influenza und PCV2 infection were the most common causes of bronchopneumonia. Over the same period, the EP infection rate dropped from 3% on < 0.1% per year.
Conclusion: Thanks to the national eradication program of enzootic pneumonia (EP) and actinobacillosis, the health status of the lungs has been considerably improved and the prevalence of pleurisy decreased notably. The results of this study indicate a good herd health in Swiss pig production.

Disclosure of Interest: None Declared

Keywords: Actinobacillus pleuropneumoniae, Enzootic pneumonia, Eradication

Herd Health Management and Economy

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Use of PADRAP- Production Animal Disease Risk Assessment Program- in 91 farms in Spain

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Introduction: One of the foundations upon which prevention, control and eradication of the diseases are based is identifying and controlling internal and external risks factors for introduction and spreading of pathogens into the farms. The PADRAP-Production Animal Disease Risk Assessment Program- was developed to support evaluation and management of risks that are predictive of clinical PRRS episodes for individual farm sites. The objective of this study was to evaluate the biosecurity level of Spanish farms using PADRAP.
Materials and Methods: In Spain 91 sow farms completed PADRAP on-line between 2014 and 2015 to assess the current biosecurity status. The results were compared to other swine farms round the world in the PADRAP database.
Commercially available software (Minitab 16 for windows) was used for statistical analyses.
Results: From a database of 91 Spanish farms PADRAP mean external and internal scores were compared with the global database of PADRAP. Both mean external and internal risk scores were higher in Spain than global scores. For internal risks the mean was 21.2 vs 19.0 and for external risks the mean was 24.6 vs 21.7.
Nevertheless a 14% of the farms had a score below the global mean external and internal risks.
There is a high dispersion in the scores of the 91 Spanish farms for both external and internal risks. The score for external risk was between 14, 6 and 38. The 50% of the farms were between a score of 22, 4 and 29, 1. The score for internal risks was between 14, 44 and 34, 4. The 50% of the farms were between a score of 18, 35 and 25 for internal risks.
Conclusion: Measuring external and internal risks is necessary to improve the understanding and management constraints that affect in a PRRS control program. PADRAP is a tool to measure risks as well as rank the farms. A correlation between less score and lower occurrence of PRRS outbreaks has been demonstrated.
In Spain we still have several farms which are above to the global median scores and the dispersion is very high, so we still have to go on making more efforts to improve biosecurity in farms with high scores, mainly in high pig density regions.

Disclosure of Interest: None Declared

Keywords: PRRS control program, risk assessment

Poster Abstracts

Herd Health Management and Economy

PO-PT2-230

Patterns of mortality in the finishing stage on 3 experimental pig farms

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Introduction: Mortality and its evolution in pig finishing has received little attention compared to pre- and postweaning mortality. Pigs dying at an older age and weight represent, however, a higher investment and value compared to early mortalities. As such, knowledge about pig mortality chances at different ages may influence management decisions. To get an idea of 1) the pattern of mortality in function of bodyweight, 2) the variability of mortality over years, and 3) the differences between farms, we compared mortality records for three experimental farms over 4 years.
Materials and Methods: The farms differed in stocking strategy: farm A sold the majority of its piglet production and finished the remaining piglets while farms B and C bought its piglets from a supplier. Cumulative mortality curves in function of bodyweight were constructed per year per farm. For all farms, third degree polynomials were fitted to the cumulative mortality curves.
Results: The form of the functions differed between farms A: $y = 8E\text{-}05x^3 - 0.0262x^2 + 3.2759x - 52.724$; farm B: $y = -0.0002x^3 + 0.0461x^2 - 1.8957x + 26.208$; farm C: $y = -6E\text{-}05x^3 + 0.0175x^2 - 0.5312x + 10.032$ with y the cumulative mortality and x bodyweight (kg).Mortality between 20kg and slaughter amounted to 4.55% (2.64 – 6.01% per year) for farm A, 1.05% (0.64 – 1.94%) for farm B, and 1.11% (0.60 – 1.92%) for farm C.On farm A, 25% of the piglets died before 30kg, 50% before 46kg and 75% before 69kg. On farm B, this was 53kg, 70kg and 85kg and on farm C, 56kg, 81kg, and 103kg. While the form of the function was relatively constant over the years in farm A, this was less for farm B or C. A reason may be the difference in total absolute mortality. Less mortalities on farm B and C, increase the influence of one mortality on the function's form. The difference in total mortality between these farms is large. We hypothesize that stocking strategy may play a role. When selling piglets, it is important to have uniform and healthy groups of piglets. Therefore, aberrant piglets may not be sold and might have a higher risk of dying. This might coincide with the higher percentage of dead pigs at a lighter bodyweight. In contrast, a finishing farm may benefit from selecting healthy piglets, diminishing the risk that pigs die during finishing. This hypothesis needs testing on a larger set of farms.
Conclusion: Based on the observed mortality figures in these farms, we conclude that the pattern of mortality in function of bodyweight is subject to differences between farms and years. However further research needs to investigate the use of such mortality curves in management support systems.

Disclosure of Interest: None Declared

Keywords: Curves , finishing pigs, Mortality

Herd Health Management and Economy

PO-PT2-232

Weaning in the farrowing pen – a potential way to reduce the use of zinc oxide and antibiotics

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Introduction: By letting the pigs stay in the farrowing pen at weaning they will not need to adapt to a new environment and they will remain in the same group of littermates as during suckling. This is expected to make the weaning process less stressful and thus improve production results and lower the need for in-feed zinc oxide and antibiotics.
For the last 2-3 years, SEGES Pig Research Centre and manufacturers have worked on developing a farrowing pen with well-functioning feeding equipment where the pigs can remain 5-7 weeks after weaning. The function of such a pen and the production results were evaluated in a commercial herd.
Materials and Methods: The trial was conducted in a pavilion with only eight pens and all-in all-out management. The pen was equipped for a loose lactating sow and measured 2 x 3 m. The feeder was a modified tube feeder (FunkiMat) selected for the trial because preliminary testing confirmed it to be the most appropriate feeder for sow and pigs.
The function of the feeder and the pen were examined during four batches of lactating sows for four to five weeks and the weaned pigs for six weeks post-weaning. Each batch consisted of averagely 93 pigs. The pen function was examined by recording pen fouling. Average daily gain after weaning was recorded pen wise.
After weaning the pigs got feed without extra added zinc oxide and did not get any group treatment with antibiotics.
Results: Only small amounts of fouling were seen in the pens.
The feeder was fully functional for both sow and pigs if the tube and dosing device are combined with a trough with a size of for instance 60 x 35 cm, the pendulum is dismantled during lactation and water in the trough is shut off once pigs are weaned.
On average the weaner pigs gained 473 g/day from 7 kg to 30 kg and the mortality rate was 2.7%. Only 2-7 pigs per batch needed individual medical treatment. Weaners housed in traditional units of the herd had a daily gain of 406 g and a mortality rate of 2.0% in the same period. These pigs received high doses of zinc oxide (2500 ppm added) and group treatment with antibiotics was used.
Conclusion: The trial showed that it is possible to develop a pen and feeding equipment that can be used for both lactating sows and weaned pigs. It was also possible to wean the pigs without using high doses of zinc oxide. The promising results are probably due not only to weaning in the farrowing pen but also to the litter-wise housing and the small batches.

Disclosure of Interest: None Declared

Keywords: farrowing pen , Pen design, weaning pigs

Herd Health Management and Economy

PO-PT2-233

RETURN ON INVESTEMENT OF A RESTRICTED INTRADERMAL PRRSV MLV MASS-VACCINATION (PORCILIS PRRS® WITH IDAL®) IN A CLINICALLY INFECTED HERD IN BRITTANY

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Introduction: A new restricted intradermal PRRSv MLV mass vaccination protocol of a 260-production farrow-to-finish farm in Brittany has resulted in an important economical benefit.
In April 2015, despite of an enhanced vaccination program of the breeding herd (2 MLV + KV at each reproductive cycle), PRRSv circulation persisted in growing pigs (confirmed by serology in 80-day old pigs) and caused coughing, poor growth, ear necrosis, poor feed conversion, anorexia and high mortality-rates.
Materials and Methods: A restricted mass vaccination (all breeding animals + all piglets from 2 to 12 weeks old) and sectoring of weaning and fattening units were implemented at once. Thereafter, all breeding animals were vaccinated every 15 weeks, piglets were vaccinated at weaning and gilts were vaccinated at arrival and 4 weeks afterwards. All vaccinations were done intradermally: Porcilis PRRS® with IDAL® injector. The farmer considered the new protocol as very practical because of 1) the user friendly IDAL® injector and 2) the restrictions of the initial mass vaccination.
Results: A reduction of clinical signs was seen from the first vaccinated batch on. A comparison of the six months before and the six months after the implementation of the new protocol showed an important improvement of the technical results. Pig production per sow per year was increased by 1,5. The ADWG of growing pigs was increased by 33g/d from 8-30kg and by 12g/d from 30-115kg. The FCR of growing pigs was reduced by 0,23 from 8-30kg and by 0,28 from 30-115kg. Age at slaughter was reduced by 5 days.
Conclusion: An economical calculation with fixed prices of both feed and carcass (prices 2014-IFIP®) showed an increase of the margin per sold pig of 12€. This economical benefit is remarkable, taken in consideration the fact that only half of the sold pigs in the period 04/15 to 09/15 had been vaccinated! In this field case, an ROI of 10 was obtained.

Disclosure of Interest: None Declared

Keywords: PRRS, ROI, Vaccination

Herd Health Management and Economy

PO-PT2-234

Attempted Eradication Of Prrs Virus, Enzootic Pneumonia, Actinobacillus Pleuropneumonia And Streptococcal Meningitis

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Introduction: The farm was a closed herd using AI and comprised 430 breeding sows. Progeny were kept until 10 weeks of age when they were sent to a second unit for finishing. The farm was infected with PRRS virus, as well as enzootic pneumonia (EP) (*Mycoplasma hyopneumoniae*), pleuropneumonia (*Actinobacillus pleuropneumoniae*) (APP) and streptococcal meningitis/arthritis (SM) (*Streptococcus suis*). The farm was planning to replace the weaning accommodation hence an opportunity arose to depopulate the farm's growing animals and to focus on eradication procedures in the breeding stock. The finishing site was also depopulated.
Materials and Methods: Gilts at 6 months of age were stockpiled from the finishing house prior to the start of the programme. They were vaccinated against *M. hyopneumoniae* and given tulathromycin (Draxxin® – Zoetis) to resolve ongoing respiratory infections and encourage lung lesions to heal. All of the breeding stock were injected with a killed PRRSV vaccine (Progressis® – Merial) initially and this was followed at monthly intervals with two live PRRSV vaccinations (Porcilis PRRS – MSD). When all the new gilts were 10 months of age, the whole breeding herd was treated with tiamulin (Denagard®– Elanco) in feed at 10mg/kg bwt for 4 weeks to eliminate *M. hyopneumoniae*. As the strain of APP was shown to be resistant to macrolides, marbofloxacin (Forcyl® – Vetoquinol) by injection was used to eliminate the organism in the last week of medication. A further two weeks in-feed medication was given containing trimethoprim/sulfadiazine (Trimediazine® – Vetoquinol) at 15mg/kg bwt to support the elimination of APP and possibly *S. suis*. Piglets were injected with tulathromycin on a weekly basis and weaned off site until the piglets from the tiamulin and TMP/S medicated sows came through. Piglets were monitored for PRRSV and *M. hyopneumoniae* by PCR, using saliva and pooled blood samples. Lungs were regularly checked for lesions at slaughter, at approximately 6 month intervals.
Results: Lung lesion scores were compared from before (18/9/2013) and after the programme (19/6/2015). EP-like lesion scores had fallen from 4.91 to 0; APP lesions had fallen from 6.8% to 0%; pleurisy had declined from 36.7% to 7.9%; pericarditis had increased from 2% to 5.9%. The *S. suis* infection rapidly returned and still required treatment.
Conclusion: Three common respiratory infections (PRRSV, EP & APP) were eradicated by focussing on vaccination and treatment of the breeding herd. Biosecurity was also improved by restricting access to the site of vehicles and personnel, to try to prevent re-infection and the programme has been successful for the last 18 months.

Disclosure of Interest: D. Burch: None Declared, B. Bremner: None Declared, C. O'Neill Conflict with: Employee, U. Klein Conflict with: Employee

Keywords: Enzootic, Eradication

Poster Abstracts

Herd Health Management and Economy

PO-PT2-235

BioChek Diagnostics Software: the 24/7 Link between Laboratory and Practitioner

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Introduction: BioChek II Diagnostics Software connects sample submitters with diagnostic laboratories. These laboratories can be independent or belong to an integrator group or veterinary clinic. The laboratory and the sample submitter should have the BioChek II Software installed. The software system uses a cloud to communicate with any device connected to the internet. The software generates a bar-coded submission form, which is used by the submitter and contains fixed data like name, address and (unit of a-) farm. Variable data like requested assays are filled in manually. The barcode is scanned by the laboratory and the fixed data is transferred. When the requested assays are performed and released the submitter receives a notification and has direct access to the data on a 24/7 basis. The BioChek II Software generates tailor made reports in which historical data can be in-cooperated.
Materials and Methods: As a proof of principle the PCV2 situation on a multiplier farm was studied. The suspicion was that a PCV2 vaccination at 3 weeks of age was not giving protection. The farm was producing their own future breeding stock and applied a high replacement rate. Gilts and sows were not vaccinated. Different age groups present at the farm were sampled. The samples were analyzed by BioChek BV, the Netherlands using the BioChek PCV2 Antibody ELISA Test Kit and the BioChek PCV2 qPCR. Data are presented using the BioChek II Software.
Results: The piglets at 3 weeks of age (moment of vaccination) had a mean titer of 4535 with a %CV (co-variation) of 65. At 7 weeks of age the mean titer decreased to 2465, clearly indicating that no seroconversion after vaccination had occurred. At 4 months of age the mean titer was 1667. At 7 months of age the mean titer had risen to 4707, indicating a PCV2 field virus infection. In the gilt population the recorded mean titer was high (6743) and uniform (%CV 8). In the sow population the mean titer was 5338.
Conclusion: No seroconversion was seen after PCV2 vaccination. PCV2 virus infection occurred between 4 and 7 months of age leading to high antibody titers in the gilts. Due to the high replacement rate a large number of piglets originate from gilts. The high levels of maternally derived antibodies in these piglets interfered with the vaccination. The PCV2 field infection occurring during the period that protection by vaccination was expected, is most likely the cause of the situation on the farm. BioChek II Diagnostic Software provides an essential tool in modern herd health management. Both ELISA and qPCR results can be displayed simultaneously, and the report style can be chosen to suit the needs of the user.

Disclosure of Interest: None Declared

Keywords: BioChek , Diagnostics , Software

Herd Health Management and Economy

PO-PT2-236

DANISH Transport standard

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Introduction: In order to maintain a high standard of health, animal welfare, meat safety and traceability in Danish swine herds, a private 3rd party audit scheme called the DANISH Product Standard was established in 2007. In 2010 DANISH Transport Standard (DANISH) was established. The main purpose of DANISH is to keep Denmark free from unwanted diseases in hoofed animals. Denmark is highly dependent on pig exports, and would suffer extensive economic losses should an outbreak of disease occur.
Materials and Methods: The DANISH Standard is a voluntary scheme which addresses pig herds, hauliers of pigs and cattle, exporters and DanAvl distributors, cleaning and disinfection stations, and collection centres. Approved DANISH hauliers are required to use DANISH cleaning and disinfection stations before loading and transporting from DANISH approved herds. Countries outside the EU, countries with borders out of the EU, and countries with certain notifiable diseases from the OEI-list are all considered high risk countries. In Denmark it is a requirement to register all transports of pigs and cattle with the Central Husbandry Register (CHR). This transport data is merged with a database at the cleaning and disinfection station to verify DANISH compliance. EU-vehicles performing long transports are monitored via GPS, and will undergo an imposed 48- hour quarantine in Denmark if GPS data cannot document and verify that said vehicle has entered only low risk countries within the last 7 days. The quarantine is also imposed if a vehicle has entered any foreign country prior to a national transport. DANISH requires that all vehicles have valid cleaning certificates, and that quarantines are met, before vehicles approach a herd. DANISH approved herds are not allowed to receive animals from non-DANISH approved herds, nor deliver to a non-DANISH approved collection centre. In order to insure compliance, a monetary penalty will be imposed to participants who do not meet these DANISH requirements.
Results: The majority (95%) of the pigs produced in Denmark are included in the scheme. Participation increases revenue to farmers and decreases the risk of fatal disease outbreaks.
Conclusion: The implementation of the DANISH Transport Standard, which requires additional cleaning and disinfecting of cattle and swine transport vehicles traveling from outside Denmark to Danish livestock herds, insures a low level of dissemination from vehicles. In addition, the 48-hour-quarantine further reduces the risk of dissemination from high risk countries. In other words, DANISH Transport Standard insures low risk of dissemination from foreign countries to Denmark, and between Danish herds.

Disclosure of Interest: None Declared

Keywords: biosecurity, Desinfection of vehicles, Quality assurence scheme

Herd Health Management and Economy

PO-PT2-241

Towards the use of Precision Farming as a decision making tool in Animal Health: A French case.

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Introduction: Precision Farming is based on the collection and analysis of farm data with the aim to optimize the farm management operations and thus to improve the returns. In swine production, usually the diagnosis and the control of diseases are based on clinical observations associated with laboratory investigations. In the case of a subclinical form, some diseases can be difficult to diagnose and the impact of a control measure is difficult to assess. However these infections can negatively impact the pig's performance. In such cases, the knowledge of economic farm data can be helpful. In the present study Precision Farming was applied to measure the impact of an intervention for the control a subclinical PCV2 infection in a French farm.
Materials and Methods: The study was conducted in a 210 sows farrow to finish farm, positive for M. hyo and Lawsonia intracellularis, both controlled by vaccination. The farm appeared to be clinically healthy as no clinical symptoms were observed but the economical performances were not fully satisfactory. The farmer and the veterinarian suspected PCV2 to be involved. To objectify this, the veterinarian sampled 9 animals, 3 per age class: 75, 100 and 140 days of age. All samples were tested negative by PCR. At a later time point serum samples were taken at 75, 100, 140 and 165 day of age and tested with an ELISA of anti-PCV2 antibodies. A seroconversion was observed from 140 days of age. Based on these results, it was decided to start to vaccinate against PCV2 (Ingelvac CircoFLEX®, 1ml I.M.) at 28 days of age and to monitor the economical performances using the batch data available. In total 2105 piglets from 6 batches were followed up.
Results: The Average Daily Gain (ADG) was 709 g/day before vaccination with Ingelvac CircoFLEX® and 732 g/day after vaccination. The difference in ADG before/after vaccination was significant ($p<0.01$). In addition, the Feed Conversion rate improved significantly from 2.70 kg/kg to 2.61 kg/kg between the period before and after vaccination with Ingelvac CircoFLEX® ($p>0.01$). Finally, the mortality rate was significantly reduced (6.54% before versus 3.88% after, p=0,006). The margin per pig was improved of €4.09 considering a feed cost of €255 / ton of feed and €1.33 per kg of carcass weight.
Conclusion: This study demonstrates that a subclinical PCV2 infection can have a negative impact on performance parameters. In the case of a subclinical PCV2 infection, the veterinarians together with the swine farmers can use different tools to assess the economic performance of the farm for diagnostic purposes but also to monitor the efficacy of the control measures they implement.

Disclosure of Interest: None Declared

Keywords: Economic benefit, PCV2 vaccine

Herd Health Management and Economy

PO-PT2-246

CORRELATION OF DIFFERENT APPROACHES TO DIAGNOSIS OF PORCINE PNEUMONIA IN NIGERIA

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Introduction: The pig is a prolific and fast growing livestock, but certain factors limit its production and utilization in developing countries including Nigeria. Respiratory diseases are of considerable economic importance however, less emphasis is on the diagnostic approaches in porcine health in our environment. The aim of this study is to correlate the haematology, gross, morphometric, bronchoalveolar lavage BAL and histopathological changes associated with pneumonia in pigs slaughtered in Ibadan, Nigeria.
Materials and Methods: The study was conducted in Bodija abattoir for over three months. Physical and clinical examination was done for each pig while different parameters such as age, sex, breed and body condition of the animal was duly recorded. Blood samples were taken after stunning for haematology using haemocytometric techniques. A total of 146 plucks were examined and assessed for lung lesions. Gross assessment of pneumonic lesions was made as percentage of lung tissue; samples were taken accordingly for routine BAL and histological examination using standard techniques. Data was summarised as percentages and M±SEM, and compared at 5% significance.
Results: Six breeds were slaughtered; large white (76.7%), mixed breed (10.3%), duroc (4.8%), local (4.1%), Hampshire (2.7%), large black (1.4%). Most of the pigs were finished pigs above 7months old. 71 (48.6%) were males while 75 (51.4%) were females. Grossly, 57.5% were pneumonic with a mean consolidation score of 15.7±1.7 ($p<0.05$). The large white breed had the highest consolidation score. Also, the right and left caudal lobes had the highest percentage lung consolidation. There was mild anaemia and leucocytosis, BAL fluid cellular differential showed increased lymphocytic and neutrophilic counts for the pneumonic pigs ($p<0.05$). The histological patterns include; broncho-interstitial pneumonia (45), bronchopneumonia (22), parasitic pneumonia (2) and granulomatous pneumonia (1), atelectasis (2), pulmonary congestion and oedema (12) and normal (62). There was a strong correlation between BAL and morphological diagnoses.
Conclusion: Porcine pneumonia is still significant in our environment; with broncho-interstitial pattern the most prevalent. This study showed that all diagnostic methods used were useful in diagnosis of porcine pneumonia in a poor resource setting with a strong indication however, for molecular and immunohistochemical techniques. These diagnostic measures will help in arriving at the possible role of different causal agents in the pathogenesis of porcine pneumonia thereby providing the basis for production of intranasal vaccines capable of curtailing porcine pneumonia in Nigeria and other parts of West Africa.

Disclosure of Interest: None Declared

Keywords: Diagnostics, Pneumonia, Swine

Poster Abstracts

Herd Health Management and Economy

PO-PT2-248

Trials to control biofilm and microbiological water quality in water pipe systems of flatdecks by the water hygiene biozide Virbac Clean Pipe (VCP).

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Introduction: Animal drinking water is the most important feed. According Regulation (EC) No. 183/2005 add. 3 it has to be offered in sufficient volume and suitable quality. There are no legally fixed requirements to define suitable quality. But the German government had given guideline parameters for animal drinking water according to already existing human legal regulations. At the dew ponds (nipple, trough) the microbiological criteria are often not achieved. Beside cases of already initial charges of the water source, biofilm development challenges all drinking systems microbiologically. The material and the course of the water pipes beside low water flows and high temperatures are only a few factors influencing biofilm development. The biofilm could impacts animal´s health by endotoxin release, but could be a source of obligate and facultative pathogens as well. Because 95 % of all microorganisms are located in the biofilm, only water sampling will underestimate the potential animal health risks.
To assess the all over disinfecting activity of a novel water conditioner and to confirm its long lasting effect of water quality improvement by biofilm control, a field trial was performed to sample water and biofilm as an unit.
Materials and Methods: The trials were done in a flatdeck unit with separate water source. The drinking water was conditioned continuously by 0,002 % of a patented Na-Hypochlorit-Formulation (VCP, Virbac) in compartment 1 at first. A similar constructed compartment 2 was left as unconditioned control. After 9 samplings in 14 days intervals the compartment conditioning was reversed. The disinfection measurements were done in removable duct segments (25 cm), ball valve closed and incorporated at the start and the end of the PVC-water pipe system. Water and biofilm inside the removed duct segment were tested microbiologically and dry mass was quantified. At each sampling additionally, a water source sample and a drinking nipple water sample were tested microbiologically.
Results: The reduction of already existing biofilm and the reduced development of new biofilm were shown when using VCP. During the water disinfection by VCP, the total mesophilic flora (36°C) was reduced to less than the critical value of 1,000 CFU/ml. Compared to the non-disinfected control group, no E. coli were found in VCP conditioned water.
Conclusion: Virbac Clean Pipe (VCP) is a practical tool to control biofilm development in water pipe systems shown exemplarily in the challenging flatdeck situations. It is an excellent disinfectant to improve drinking water quality and to reduce pathogens in water for animal consumption.

Disclosure of Interest: None Declared

Keywords: Disinfection, E.coli, Hypochlorit

Herd Health Management and Economy

PO-PT2-251

Modelling the economic efficiency of using different strategies to control Porcine Reproductive & Respiratory Syndrome at herd level.

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Introduction: In recent years, mathematical models have been increasingly used as tools by farmers and veterinarians to estimate the cost of diseases and the economic efficiency of different control strategies. Recently, a model estimating the costs of Porcine Reproductive & Respiratory Syndrome (PRRS) at individual farm level was developed. Since different control options such as vaccination and elimination protocols are available for combating PRRS, the aim of this study was to provide a tool to model the economic efficiency of different strategies to control PRRS at individual farm level.
Materials and Methods: The baseline model used, a stochastic spread sheet model, estimates the effect of PRRS infection on health and productivity in a specific industry setting (different farm types, herd sizes, types of batch farrowing etc.). In this model, different intervention scenarios were incorporated: a) depopulation/repopulation, b) close & roll-over, c) test & removal, d) mass vaccination of sows, e) mass vaccination of sows and vaccination of piglets, f) vaccination of sows according to the status of reproduction (6-60), and g) vaccination of sows (6-60) and vaccination of piglets. Additionally, the improvement of internal biosecurity was accounted for. Interventions d) to g) were considered alone and in combination with the improvement of biosecurity. Corresponding costs for all measures can be entered by the user. Data on reduction of PRRS disease parameters for each type of intervention and data on probability of success were obtained through literature review and expert opinion.
Results: Economic efficiency of the different control strategies is assessed over a period of 5 years through investment appraisals. These estimate the present value of extra costs and extra revenues due to the interventions. The final net present value is multiplied by the probability of success of each intervention, and the expected value obtained is used to indicate the most cost-effective strategy. The model output that includes information about the costs of PRRS on the particular farm is completed by the payback period, the cost-benefit ratio and the internal rate of return. All results are displayed separately for every intervention measure that can be applied on the particular farm.
Conclusion: The presented model gives new insights regarding the efficiency of various control measures currently available to combat PRRS at herd level. Although often propagated in the field, not all control and prevention measures for PRRS have been proven to be economically efficient in all cases. The model facilitates the estimation of personal consequences for a specific farm setting and thus enables a better informed decision.

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Keywords: Efficiency of interventions, Farm level calculator, PRRS

Herd Health Management and Economy

PO-PT2-255

REVALUATION OF A DIGESTIVE ORAL ANTIBIOTIC TREATMENT IN A POST-WEANING UNIT USING IPC®

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Introduction: Prudent use of antibiotics implies rational use of the available medication and their periodic revaluation regarding the ever-evolving situation of a particular herd. In this case the prescriber vet uses the data management tool Individual Pig Care® to monitor the removal of the digestive in-feed antibiotic treatment to pigs 6-to-10 weeks old.
Materials and Methods: IPC® translates the continuous records into graphical reports using the same time-line. These reports being accessible on-line and constantly updated, the veterinarian can follow "live" and at distance the evolution over time of each valuable data he has selected for a given herd. The revaluation of the antibiotic treatment is divided into three steps: 1/ initial situation: for two consecutive batches, IPC® compiles daily records at individual and batch level: clinical symptoms, morbidity, mortality, room temperature and hygrometry, individual and collective treatments. 2/review of the critical points: the monitoring allows the veterinarian and the farmer to rank and implement corrective actions: adjustment of the ventilation system, replacement of Ingelvac Circoflex® by Suvaxyn PCV® and removal of the in-feed antibiotic treatment. 3/ impact evaluation of the treatment removal and other identified risk factors by recording the same data on the next two batches.
Results: Morbidity and mortality criteria are improved; individual and collective treatments are decreased. The revaluation shows the antibiotic treatment can be objectively removed without any negative impact on the clinical status of this herd.
Conclusion: IPC® proves to be an effective, adequate and easy-to-use management tool for the clinical revaluation of an antibiotic treatment in a commercial herd, converting the farmer's daily observations into an objective assessment of the pig health. Combined with the zootechnical performances records, IPC® allows an extensive economical calculation and forms a complete data management system.

Disclosure of Interest: None Declared

Keywords: antibiotic use, post weaning diarrhoea, revaluation

Herd Health Management and Economy

PO-PT2-256

Successful Attempt To Eradicate Prrs Virus And Enzootic Pneumonia From A Breeder/Finisher Herd

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Introduction: The farm comprised 450 breeding sows plus finishers, all on one site. The farm was infected with PRRS virus, against which sows were not vaccinated, enzootic pneumonia (EP) (*Mycoplasma hyopneumoniae*) as well as pleuropneumonia (*Actinobacillus pleuropneumoniae*) (APP) and Glässer's disease (GD) (*Haemophilus parasuis*) but no clinical *Streptococcus suis*. The farm was planning to replace the growing accommodation and thought this would be a good opportunity to depopulate the growing side of the herd and focus on the breeding herd to eradicate some of the endemic diseases present, PRRSV and EP and hoped this would then alleviate the APP and GD.
Materials and Methods: Gilts at 6 months of age were stockpiled from the finishing house prior to the start of the programme and reared off site. They were vaccinated against *M. hyopneumoniae* and injected with tulathromycin (Draxxin® – Zoetis) to resolve any respiratory infections. All of the breeding stock were vaccinated with a live PRRSV vaccine (Porcilis® PRRS – MSD), which was repeated one month later and followed with a killed PRRSV vaccine (Ingelvac® PRRS KV – Boehringer Ingelheim). When all the new gilts were 10 months of age, the whole breeding herd was treated with tiamulin (Denagard®– Elanco) in feed at 5mg/kg bwt plus chlortetracycline (Aurofac® - Zoetis) 15mg/kg bwt for 4 weeks to eliminate *M. hyopneumoniae*. Piglets were injected with tulathromycin (Draxxin® – Zoetis) on a weekly basis and weaned off site. Piglets were monitored for PRRSV and *M. hyopneumoniae* by PCR, using saliva and pooled blood samples. Once cleared, the piglets were then weaned back on site. Lungs were checked for lesions on a regular basis at slaughter.
Results: Lung lesion scores were compared from before (23/1/2014) and after the programme (3/11/2015). EP-like lesion scores had fallen from 8.4 to 0; pleuropneumonia lesions fallen from 1.1% to 0%; pleurisy had declined from 22.4% to 1.5%; pericarditis had fallen from 6.7% to 1.5%. At present, no routine antibiotic use was applied during the growing period although APP has been re-isolated on the farm. GD was expected to return but has not. The same 6 month's performance data before and after the programme (April-September) were compared and mortality post weaning to slaughter has reduced from 4.7 to 3.1%, ADG improved from 626 to 765g (21.6%) and FCR from 2.62 to 2.22 (15.3%).
Conclusion: The two endemic respiratory infections PRRSV & EP were eliminated by partial depopulation of the herd and focussing on the vaccination, medication and management of the breeding herd. This has improved the performance of the growing herd and reduced the use of antibiotics, markedly. Biosecurity has also been enhanced.

Disclosure of Interest: D. Burch: None Declared, R. Grogan: None Declared, C. O'Neill Conflict with: Employee, U. Klein Conflict with: Employee

Keywords: Enzootic, Eradication, Pneumonia

Poster Abstracts

Herd Health Management and Economy

PO-PT2-257

THE IMPLEMENTATION OF A MONITORING TOOL (LARYNGEAL SAMPLING) IN A GILT ACCLIMATION PROGRAM FOR MYCOPLASMA HYOPNEUMONIAE: A CASE STUDY

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Introduction: *Mycoplasma hyopneumoniae* is one of the key contributors to Porcine Respiratory Disease Complex (PRDC). Control of PRDC will not be achieved until a proper acclimation program of the gilts to *M. hyo* before entering the breeding herd is implemented. *M. hyo v*accines can control clinical disease but cannot prevent infection so that acclimation is a critical process of the Infection Chain™ concept.
Laryngeal swabs showed the highest sensitivity for early detection of *M. hyo* compared to other sample methods.
This case study documents the use of laryngeal swabs as a monitoring tool to optimize and validate a gilt acclimation program for M.hyo.
Materials and Methods: This case study was documented in a *M. hyo* positive 3.000 sow farrow to feeder farm *M. hyo* positive, located in the central region of Spain. The gilts for this site and for another 3.000 sow farm site of the same production system, are born and raised in the site of the study. 30 randomly selected gilts were sampled at 70, 90, 112 and 132 days of age, using laryngeal swabs to assess the pattern of infection and shedding of *M. hyo*. According to the results we intended to use the age group of gilts with the highest *M. hyo* excretion rate as seeder pigs for acclimation of new arrivals in the AI/AO per room gilt development unit. The final goal of the acclimation protocol is to promote early exposure during the acclimation process.
Five months later we sampled again gilts at 75, 95 and 120 days of age to determine if there had been a change in the pattern of shedding.
We ran real time PCR in pools of 3 samples.
Results: In the first sample, with 90 days of age we found a 100% of PCR positive gilts, so we decided to use gilts of 90 days of age as seeder pigs for new arrivals entering the development unit at the age of about 55days.
In the second sample, we saw that the age with a high shedding was delayed to 120 days of age; therefore we adjusted the acclimation protocol and we used gilts of 120 days of age as seeder pigs.
Conclusion: A proper gilt acclimation program is necessary in order to control *M. hyo*. Laryngeal swabs are a good tool to assess the shedding status of the gilts to identify seeder pigs for acclimation, allowing a proper adjustment of the program. Due to possible shifts in the age groups with highest shedding status, a periodical re-evaluation of the shedding status is recommended to optimize the acclimation program.

Disclosure of Interest: None Declared

Keywords: Gilts aclimatation, Mycoplasma hiopneumoniae,Laryngeal sampling

Herd Health Management and Economy

PO-PT2-264

Lung lesion survey using Ceva Lung Program in Russia, Ukraine and Belarus

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Introduction: Lung scoring at the slaughterhouse is a valuable tool for the assessment of the respiratory health status of a large number of animals. Moreover, there is a clear relation between lung lesions present at slaughterhouse, economic impact of respiratory disease and efficacy of control programs, rendering lung scoring an attractive tool for making decisions and monitoring veterinary interventions. To facilitate efficient and hygienic lung lesion scoring at slaughterhouses, Ceva recently developed a tablet-based software tool allowing for rapid scoring, with automated processing and storage of data. This application is a part of Ceva Lung program (CLP). Information from such monitoring is very valuable to establish a real prevalence of Enzootic Pneumonia (EP) and *Actinobacillus pleuropneumoniae* (A.p) on a farm- or even a national level.
Materials and Methods: In between January 2015 and June 2015, a total of 117 batches consisting of 9253 lungs were scored using the CLP app. Lungs were scored following the CLP method for the presence, type and extension of lung lesions described as: - Enzootic pneumonia (EP)-like lesions following a modified Madec methodology; Cranio-ventral pleurisy (score 0-1); -Scarring, describing the prevalence of fissures associated with older EP-like lesions (score 0-1); Dorso-caudal pleurisy score, to describe A.p - like lesions (scale 0-2-3-4), Actinobacillus pleuropneumoniae Index (APPI), showing the prevalence and extension of dorso-caudal pleurisy. Lungs were scored from farms originating from Russia (58 batches), Ukraine (44 batches) and Belarus (15 batches).
Results: ukraine and Belarus showed high % of affected lungs by M. hyo like lesions: 64% and 52% respectively (expressed as median) and 30 % of examined lungs in Russia showed M hyo like lesions. The highest % of lungs affected by A.p like lesions were evaluated in Belarus and Russia: 52 and 42% respectively (expressed as median). The APPI index ranged between 0.41-1.41; 0-0.63 and 1.02-1.79 in Russia, Ukraine and Belarus respectively.
Conclusion: EP - like lesions have a high prevalence in pig lungs investigated in all three countries with relatively high scar score in Russia and Belarus suggesting early infection of lungs. Interestingly the incidence of A.p -like lesions was relatively low in Ukraine, both Russia and Belarus had high incidence of A.p like lesions, actually almost every second lungs were affected on averge. Using the Ceva Lung Program, it is possible to easily and correctly assess the incidence and severity of lung lesions attributed to previous M.hyo and A.p infections.

Disclosure of Interest: None Declared

Keywords: Enzootic pneumonia, lung lesions, Pleuropneumonia

Herd Health Management and Economy

PO-PT2-266

Effect of medium chained fatty acids on the use and effectiveness of antibiotics

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Introduction: Finding a balance between reducing the amount of antibiotics in animal feed and breeding animals on a healthy and profitable way will be the biggest challenge for the coming years. In the near future there will be a high demand for replacement or enhancement of antibiotics with healthy and natural additives. The aim of this trial was to see if a mixture of pure and well defined medium chain fatty acids (MCFA) can be a help to install this balance on a natural and profitable way.
Materials and Methods: In an Australian trial facility the technical and economical results of three groups of 140 weaned piglets were compared after 4 weeks of treatment. Treatment 1 (T1) received standard in water antibiotics (amoxycilline/lincomycine-spectinomycine), treatment 2 (T2) received the same antibiotics together with 0.2% MCFA in the diet, and treatment 3 (T3) received only MCFA (0.2%) in the diet without in water medication. All diets where wheat based and contained ZnO (3kg/ton). Fumaric acid was used as a pH-reducing acid in the diet.
Results: There was no significant difference in daily gain between T1 (242g/d) and T3 (235g/d) while T2 showed a significant ($p<0.05$) higher growth (278g/d) when compared with the other treatments. Looking at feed conversion ratio, a significant difference ($p<0.05$) can be seen in both treatments (T2 & T3) which received MCFA compared to T1 which only received the in water antibiotics. A 7% mortality rate was seen in T1 which received only antibiotics while no mortality was seen in T2 and only 1% in T3. Economically there is a return of investment of MCFA in T2 of 13, in T1 the ROI is even 35
Conclusion: Adding MCFA on top of antibiotics leads to significant higher growth and lower feed conversion ratio. Equal growth at a significant lower feed conversion ratio was seen by replacing the antibiotics with MCFA. Both MCFA treatments have led to much lower mortality. It can be concluded that MCFA can be used to replace preventive antibiotics with better technical results. When MCFA is used on top of antibiotics this will enhance the antibacterial effect and lead to lower mortality figures combined with better technical results. This means healthier piglets that will need lower amounts of curative antibiotics. Not only will adding MCFA lead to better performances, there is also a clear economic benefit for the farmer with a ROI of 13 (T2) up to 35 (T3).
In this trial MCFA proved to be a valuable alternative for the preventive antibiotics in the weaning phase

Disclosure of Interest: K. Lannoo Conflict with: Nuscience, J. Vande Ginste Conflict with: Nuscience, W. Naeyaert Conflict with: Nuscience, R. Goedegebuure Conflict with: Nuscience

Keywords: additive, antibiotic resistance, antibiotic use

Herd Health Management and Economy

PO-PT2-267

A study of three different parameters of feeding behavior, of sows in large group gestation with ESF.

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Introduction: Animal welfare, has been traditionally evaluated by measuring level of aggression and stress parameters. However, feeding behavior can also provide a valuable indication of comfort or stress for gestating sows in large groups, when fed with ESF systems.
Materials and Methods: The study was conducted at Albesa-Ramadera a 3300 sow, Site 1 farm, based in Catalonia, Spain. The farm has large group gestation (128 to 175 sows per group) and utilizes Electronic Sow Feeding Stations (ESF). (Compident 7®, Schauer Agrotronic GmbH). Nulliparous sows are placed separated in dynamic pens, while all other multiparous sows, (parities 2-7) are placed in larger dynamic groups. All pens have two ESF, per pen. For this study, the ESF opened at 00:10, and closed at 23:50. Recording was done at 2:00 PM, on 5 consecutive days.
Average daily feeding duration AFD (in minutes) was recorded. Average number of productive entries (ANPE) records only the entries in which feed is administered.
The percentage of sows that have completed their projected feed consumption within 14 hs. (PSF-14) from start of the feeding cycle, was also recorded.
During the one week period of this study, the farm had, what could be considered average production parameters considering its present health status and genotype.
Results: When looking at AFD, sows in the different pens spent on average, from 8,49 to 13,31 minutes to eat their feed.
It can be observed that nulliparous sows took longer (approximately 13 minutes) to eat, than higher parity sows, which average around 9 minutes.
It can be observed that at least 94 % of multiparous sows had completed their meal by 2:00 PM, while just over 88% of nulliparous sow had completed their meal by that time. Sows that had not eaten by then, still had 9,5 hours remaining to complete their meal, before the ESF closed down until that next day.
Regarding ANPE, it can be observed that sow chose to eat their whole feed ration in one (0,98-1,03) feeder visit per day. Some sows, may choose to not eat for a day.
Conclusion: AFD, PSF-14, ANPE may be affected by: Portion size (dependent on the day of the feeding curve), amount of water administered with the feed, feeding rate of the sow, time parameterization of feed portions and training level of sows.
The parameters described here may be useful for evaluating normal or abnormal feeding behaviors especially in within-farm comparisons. However they should not be considered universal, since, large differences are observed when considering other types of EFS, different degree of sow training, group size, parity structure, time in gestation or amount of feed.

Disclosure of Interest: None Declared

Keywords: Electronic sow feeding behaviour

Poster Abstracts

Herd Health Management and Economy

PO-PT2-276

Yearly and seasonal evolutions in performance parameters of sows in three European countries in 2011-2013

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Introduction: Performance parameters of sows are commonly used to monitor reproductive performance, and to assess the economic viability of sow herds. In general, the efficiency of reproduction in breeding herds has significantly improved over the last decades. The aim of this study was to assess the situation on performance in sow herds in three European countries for the years 2011, 2012 and 2013.
Materials and Methods: Data were provided by three European countries (A-C). In every country, data were obtained from maximum 50 sow herds (min. 43, max. 50 herds per country). Performance parameters were collected from herd management software in countries A and B, from national health registers in country C. The number of liveborn and stillborn piglets, and the percentage of preweaning piglet mortality were investigated. Evolutions were studied per year and per month.
Results: The average herd size was 324 sows (min. 35, max. 1502). The overall averages for liveborn piglets, stillborn piglets and preweaning piglet mortality were, respectively: 13.4, 1.2 and 13.1% (A), 11.4, 1.9 and 14.5% (B), 13.9, 1.1, 13.1 (C). In country A, the average number of stillborn piglets per litter was significantly lower in 2011 (1.1) than in 2012 (1.2) and 2013 (1.2). The average piglet mortality was significantly higher in November (14.0%) compared to June (12.6%). In Country B, no significant results were seen. In country C, the average number of liveborn piglets in 2013 (14.1) was significantly higher than in 2011 (13.8) and 2012 (13.9). The average number of stillborn piglets was significantly higher in July (1.2) compared to April (1.1).
Conclusion: The overall averages found in this study were comparable to the national average of 2012. Throughout the years, the majority of the indicators of performance changed with time, for the number of liveborn piglets an improving trend could be seen. However the number of stillborn piglets seemed to increase at the same time. To conclude whether the efficiency of reproduction performance improved over the years, more parameters should be studied. Concerning the seasonal evolutions, in country A a higher piglet mortality was seen in autumn, in country C a higher number of stillborn piglets was seen in summer. Possible explanations for this could be differences in climate or housing system between countries.
This work was conducted under the EU-funded PROHEALTH project.

Disclosure of Interest: None Declared

Keywords: None

Herd Health Management and Economy

PO-PT2-277

New System of Identification of Pigs in Venezuela a Pilot Study

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Introduction: Pig farms in Venezuela, individual identification a method used in breeding pigs only, but does not occur in swine production. This condition limits the traceability of pigs from the farm to the industry. Food animal traceability has been defined as the ability to maintain a credible custody of identification for animals or animal products through various steps within the food chain. In animal production the animals have to be linked to premises along with recording all animal movements (Hurnik, et al., 2008). Several countries have already implemented pig traceability systems. However, none of the foreign systems meet specific Venezuela requirements. The aim of this study was to describe new system of identification of pigs in Venezuela a pilot study.
Materials and Methods: The first part of the pilot study was aimed at providing technical and cost information about pig identification. Five farms of continuous flow of different owners located in different states of central Venezuela with a total 500 pigs were performed with QR ear tags and 500 pigs were conventionally identified by lot number of each farm. Reading the tag it was conducted during all stages of production on the farm and in the industry until the end of the slaughter line.
Results: 500 pigs identified with QR ear tags. The permanence of the tag in farm was 99%, only one lost. In industry: the conventional batch identification is maintained but not individually, with QR individual identification ear tags is maintained until the separation of the cutting head. Use of ear tags for the identification and traceability of pigs from birth to the end of the slaughter line is all possible cases. Reading the QR code of the tag has been possible at all stages, however farm requires cleaning of the tag to be read. On the slaughter line you can perform the reading of the tag since it is kept clean during slaughter.
Conclusion: In conclusion the visual ear tag with QR code was observed to be the most legible and cost effective mean to identify pigs under the Venezuela farm production conditions. To warrant the use of this technique in practice, transponder recovery requires further investigation. An effective and practical animal movement database and system can be built in Venezuela. The first three steps of building a swine traceability system in Venezuela are:1) To approve data and access requirements for a farm premises registry. 2) To standardize ear tags for hogs going to slaughter, and to aggregate information from the packing plants.3) To build a livestock premise, hog identification, and hog movement reporting system across the country to national standards.

Disclosure of Interest: None Declared

Keywords: None

Herd Health Management and Economy

PO-PT2-278

Improving productivity in growing pigs by combining specific and non-specific monitoring

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Introduction: Disease control plays a significant role when trying to improve productivity, reduce antibiotic consumption and increase welfare. Existing methods and tools to support monitoring of growing pigs are still insufficient for early identification of disease. The goal of this study was to combine non-specific clinical and production data with test results for specific infections in order to identify key parameters and trends for early detection of disease.
Materials and Methods: The study included two herds with production of finishers in all-in all-out sections. In herd A, the pigs were vaccinated against PCV2 and *Mycoplasma hyopneumoniae* (M. hyo) one week after weaning. In herd B, the pigs were vaccinated against PRRS type 2 (modified live vaccine) at 14 days of age and against PCV2 and M. hyo one week after weaning.
In each section, four focus pens were selected, and all monitoring was carried out on pen level in the focus pens. In each of the focus pens, five pigs were ear-tagged and monitored over time.
The non-specific production and clinical data included: daily measurements of water consumption, mortality and antibiotic consumption, and weekly measurements of weight gain, feed consumption, cough index, and diarrhea index. The testing for specific infections included weekly analyses of oral fluid and monthly analyses of pen samples (fecal pools) and blood samples together with extended lung examination at slaughter. The oral fluid was analyzed for influenza virus by qRT-PCR, and the blood samples were analyzed for antibodies against M. hyo, *Actinobacillus pleuropneumoniae* (AP) serotype 6 and 12, PRRS and the viral load of PCV2. The pen samples were analyzed for *E.coli* F4, *E.coli* F18, *Lawsonia intracellularis* (LAW) and *Brachyspira pilosicoli* by qPCR.
Results: At present, three batches of pigs have been included in the study on each farm. We are currently working on graphical visualization of data in order to achieve the best overview of infection profiles and trends. An example from the data already obtained is that the pigs in herd A have antibodies against PRRS at arrival, excrete LAW in high amounts eight and 12 weeks later and are infected with AP 6 and 12 before slaughter. At each sampling, 25-50% of the pigs were viremic for PCV2 at moderate to high amounts. A similar infection profile was seen in herd B, except that the pigs did not become viremic to PCV2 at any time.
Conclusion: The graphical visualization of production data, clinical observation and test results for specific infections may potentially be an important tool for farmers and vets to get an overview of each production unit. If the data are continuously updated, early identification of disease may be possible.

Disclosure of Interest: None Declared

Keywords: Growing-finishing pigs, monitoring, production parameters

Herd Health Management and Economy

PO-PT2-280

Case study: Improvement of performance results in a Dutch farrow-to-finish farm after successive implementation of different vaccination protocols

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Introduction: Vaccination is one of the tools to prevent diseases and therefore can be a useful tool in the reduction of antibiotic use. The objective of this case study was to evaluate effect of different successive interventions on the antibiotic use and performance in a finishing unit under field conditions.
Materials and Methods: This case study was performed in a closed herd of 290 sows with 2100 finishing places. The finishing unit had a history of standardized use of Tylosin to control ileitis. Despite the Tylosin during the 6-7 weeks in the finishing the uniformity and growth was reduced. Cross sectional serology revealed PCV2 and Lawsonia in this period. M hyo and PRRS were all negative. In May 2014 the farm started i.m. CircoFLEX vaccination at 6 weeks of age and ileitis vaccination at 8 weeks of age in the drinking water.
In the period of August - November 2014 more respiratory problems with periods of high mortality were observed in finishing unit. Bacteriological examination of lung material revealed a severe Pasteurella multocida infection which was assumed to be secondary to a M. hyo infection. 2 slaughterhouse checks revealed higher than usual % of pleuritis (25% and 45%), abscesses and lung lesions (31%) and Mhyo-like-lesions (42%). End of November 2014 it was decided to exchange the Enterisol for a MycoFLEX vaccination at 5 weeks of age, mixed with the CircoFLEX vaccination.
Close out technical data were collected for the different periods. Monitored parameters were ADG, mortality, feed conversion ratio and antibiotic use.
Results: The results for the 3 different management interventions are for the ADG respectively 790, 837 and 828 grams/day. The FCR developed from 2,66 to 2,45 and 2,50; with a reduced antibiotic use of 8, 5 and 2 DDD. Mortality rates decreased from close to 6,5 % in 2014 to 2,5 % in 2015. Respiratory symptoms and antibiotic use was strongly reduced in 2015. The pleuritis and lung lesions in Mhyo vaccinated fatteners decreased less than 20 %.
Conclusion: This study demonstrates that different interventions can improve performance and reduce antibiotic usage in finishing unit. When replacing a vaccination against disease A for vaccination against another disease B, Weibel showed that also the improvement of vaccination A is replaced by improvement of vaccination B, with limited extra progress in performance. In this case study Mhyo vaccine had a positive effect on mortality and carcass quality but was not able to compensate the effect of the ileitis vaccine on ADG and FCR. The clinical picture improved clearly, which was important for the farmer and the interventions resulted in a more sustainable pig production.

Disclosure of Interest: None Declared

Keywords: antibiotic use, vaccines

Poster Abstracts

Herd Health Management and Economy

PO-PT2-281

Identification and Traceability in Iberian Pigs in Montanera a Pilot Study in Extremadura-Spain

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Introduction: The Iberian native pig breed from the south west of the Iberian Peninsula is characterized by its full use of the natural meadowland resources of pastures and acorns. Feeding in the late fattening phase, a long productive cycle under extensive management, and the lipid characteristics of its meat have combined to enable the production of a high quality meat, especially for cured meat products: hams, fillet and shoulder of pork. The aims of this study was to describe a pilot study tracing the traditional Iberian pigs intended for consumers using information and communication technologies in Extremadura, Spain.
Materials and Methods: A pilot field study to a total of 55 extensive holdings of Iberian pigs in Extremadura, Spain, between July 2014-March 2015, with a total 9500 pigs were performed with QR ear tags. The geo-location was established by a GPS and verified by the SIGPAC. Track throughout the industrial chain in slaughterhouses, industrial processor and an end outlet was conducted and evaluated a total of 250 consumers. Two prototypes of tools to manage and analyze a web platform and a website developed.
Results: The results were as follows: 55 identified holdings, geo-localized, 9500 pigs QR identified with ear tags, identification of 5 industries in the area, record the origin of the pigs, carcass weight and final products, totaling 1500 hams, 1500 pallets and loins were identified with QR seals. At consumer level, the average consumption of products and by-weekly and monthly Iberian pig was determined by applying information technology and mobile communication through use of 72% was established. The visual QR ear tag was observed to be the most legible and cost effective mean to identify pigs under the Iberian traditional production conditions. An effective and practical animal movement database and system can be built in Iberian pigs in Montanera, in Extremadura, Spain.
Conclusion: In conclusion a pilot and analysis of new traceability systems Iberian acorn pork linked to Information and Communication Technologies, based on a promotion campaign and outreach to farmers/ producers, slaughterhouses, and industrial and consumer study was conducted. This project has been funded by the Programa Coinvestiga Secretaría General de Ciencia y Tecnología (Proyecto N°EI 14-0014-1).

Disclosure of Interest: None Declared

Keywords: identification, traceability, Iberian, Montanera, pigs, QR.

Herd Health Management and Economy

PO-PT2-283

Online Monitoring Pig Health in the Netherlands: a unique system

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Introduction: Since 2001 GD Animal Health in the Netherlands runs a health monitoring system of all livestock. This system was set up on request of the Dutch government and the different sectors of food production animals. Until recently the system to monitor swine health was based entirely on passive data collection. Results from post mortem investigations of pigs submitted to the animal health service were combined with information gathered during telephonic consults and farm visits. This system proved to be very suitable to detect outbreaks of new, unpredicted, or re-emerging diseases. In addition to the passive data collecting system there was need for a more proactive system to obtain more representative data of trends in time and per region regarding herd-related health issues. After pilot studies in 2004 and 2012, an online monitoring tool was launched in 2015. This monitoring is formally integrated in quality assurance systems for pig farmers and pig veterinarians.
Materials and Methods: Typically, commercial pig farms in the Netherlands are visited by a veterinary practitioner once a month. The practitioners are recording health problems using the online tool during each farm visit. Moreover, also the absence of health issues is documented. The design of the online application involves no more than five steps, namely registration of (1) age group, (2) organ system involved, (3)main clinical symptom, (4) most probable diagnosis, (5) used laboratory test to confirm the diagnosis. The application is linked to the management information system of the veterinary practice. Data are uploaded to the GD Animal Health data system. Every month practitioners receive an overview of the results of their own practice and a benchmark, which represents the results of all other participating practices in the Netherlands. Besides, maps are produced in which trends of certain health issues in specific regions are visualized.
Results: The online monitor of pig health in the Netherlands was formally launched in July 2015. In October the monthly feedback of results to the practitioners started.
Conclusion: The online monitoring of pig health in the Netherlands is a unique system. Since it is compulsory incorporated in quality assurance systems, the participation rate is expected to be high. This system provides information to pig farmers and veterinarians on regional health issues. Finally, on sector level the information can also be used to explain for instance variation in the level or the use of certain antibiotics.

Disclosure of Interest: None Declared

Keywords: monitoring online Netherlands

Herd Health Management and Economy

PO-PT2-286

Preanalytical storage conditions influence biochemical analytes in pig blood

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Introduction: This study was initiated to establish optimal routines for handling porcine blood samples in a diagnostic laboratory highly experienced with analysing small ruminant blood samples. Various trials have shown that blood exposed to different temperatures, transport and storage duration may affect analytical results. Previous studies also indicate that sample stability differs among species. The wider objective of our study was to minimize pre-analytical storage influences and ensure diagnostic validity of biochemical parameters of pig blood, collected as part of a large, ongoing feeding trial in Norway, focused on mineral nutrition.
Materials and Methods: Eleven 4-6 month-old gilts fed standard feed were included. Ten serum vials of 9 ml were collected from each animal by jugular venipuncture. All samples (n=110) were transported to the laboratory within 120 minutes after sampling, without prior chilling. The samples were processed at different time intervals ranging from 2 hours to 72 hour post-sampling. For processing, blood samples were divided into two groups and stored at either chilled (4ºC) or room (20°C) temperature. Blood was centrifuged after 6, 12, 24, 48 and 72 hours post-sampling and frozen (-20 ºC) at the same time intervals.
Ten biochemical analytes were measured. Gamma-glutamyl transferase, total protein, albumin, urea, creatinine, inorganic phosphate, glucose, and iron were assessed by colometry (ABX Pentra 400 Analyzer, Horiba). The concentrations of calcium and magnesium, were analysed by Atomic absorption (Analyst 300 Perkin Elmer).
Biochemical analytes in the reference sample were measured repeatedly (n=18) and the coefficient of variation (CV) was calculated to assess test repeatability. Sample stability processed under different storage conditions was evaluated statistically by Analysis of Variance (ANOVA).
Results: Test repeatability was within accepted diagnostic limits, e.g. glucose CV= 0.04. Results indicate that specific biochemical parameters of pigs were significantly altered depending on storage duration and temperatures. A time temperature interaction was found for e.g. inorganic phosphate (p=0.001) and glucose (p=0.001).
Conclusion: In conclusion, this study identified alterations in some porcine biochemical analytes, relevant to veterinary clinical practicioners, researchers and diagnostic laboratories involved with handling blood samples from pigs. Overall, they highlight the need for standardized techniques for optimizing diagnostic reliability of blood samples and interpretation of results based on pre-analytical conditions, such as sample storage.

Disclosure of Interest: None Declared

Keywords: biochemical, pig, blood

Herd Health Management and Economy

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Preliminary study of the biosecurity on Irish pig farms

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Introduction: Rapidly-spreading diseases, such as ASF and PED, are a major threat to pig farms, whose major weapon to prevent them or attenuate their impact is biosecurity. The Irish Department of Agriculture, Food and the Marine has stressed the need to raise awareness for biosecurity among the Irish pig production in order to protect the Irish pig sector. In this study the Biocheck scoring system was used in 10% of the Irish pig farms in a pilot study to evaluate biosecurity.
Materials and Methods: In March-April 2015, biosecurity was assessed in 30 Irish farrow-to-finish pig farms using the previously developed protocol Biocheck.UGent scoring system. Biocheck.UGent is a biosecurity benchmarking system developed by Ghent University currently used in different EU countries (http://www.biocheck.ugent.be/v4/home/). Three Teagasc advisors and three pig veterinary practitioners were asked to run the questionnaire in 4 to 6 farms of different sizes in their area thus testing the feasibility of the survey by several assessors and different contexts. Descriptive statistics were used to examine the recorded data.
Results: The average size of the sampled farms was 930 sows (range 155 to 3000). Concerning External Biosecurity, Irish farms scored higher than the EU average in all parameters, achieving a mean of 81% vs 66%, respectively. The subcategories of “Purchase of animals and semen” and the “Environment and region” obtained the highest scores, both over 90%, denoting good practices. Internal Biosecurity presented deficiencies regarding the “Farrowing and suckling period” and “Measures between compartments and the use of equipment” in which the mean scores were lower than the EU average (51% and 36% vs 60% and 42%). In this regard, the greatest deficiency was seen in two practices in particular with 90% of the farms admitting to never changing clothes between compartments and 97% never checking the efficacy of cleaning and disinfection.
Conclusion: Ireland’s geographical location and farming system was shown to be a valuable asset for external biosecurity. However internal biosecurity should be improved to limit the circulation of disease within farms. The Biocheck.UGent biosecurity scoring system was successfully applied in Irish pig farms despite a lack of training and the multiplicity of assessors using it. It is planned to expand it to 30% of the farms during 2016.

Disclosure of Interest: None Declared

Keywords: biosecurity, Ireland, pig production

Poster Abstracts

Herd Health Management and Economy

PO-PT2-291

Xin Xing° Organisation A Three Side Production With S P F Pigs

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Introduction: The SPF farm Xin Xing° with "three-side-production" in Liuzhou city in the Guang Xi Province PR China is located in a sugar land with 20 ha own sugar fields.The next pig farm is more then 10 km away.In the year 2011 for the pig production were bought 485 SPF jung sows and boars from a breeder in the US.The farm produced breeding pigs and fatteners.
Materials and Methods: In the years 2010/11 were invested 50 Mio AMB for new buildings and pigs.Now the company with "three-side-production" owns five pig farms with 8000 sows and 150 boars.Two farms are for sows,one for piglets,one for fatteners and one for boars at different places,distance 1,0 km or more, in the land. The SPF boar station with semen production only for the own sows has a specialy air filter for reducing more then 99,5% of the dust.The pig races in the farms are Long White,Large White and Duroc.To the farm belongs a feed mill with a production of 30.000 tons feed per year.For entering a farm the workers have a shower for going in and for going out.In a own laboratory were tested blood samples from the pigs with a PCR for different diseases as CSF,MFD,M.Auj., PPV, PCV2,PRRSV etc.and for cultering germs as Mycoplasma or E.coli.The breeding pigs for selling are vaccinated by the Boehringer Ingelheim° vaccines against PCV2 and PRRSV,as these pigs go to farm with conventionel health status.
Results: The organisation sells every year 150.000 pigs.Among these pigs are 30.000 breeding pigs as sows and boars.The others are for fattening and are slaughtered in the next town.Frequent veterinarian checks of the pig population ensure that the animals are kept to high hygiene and health levels.Only sporadically sows must be treated for the MMA syndrome and piglets against early diarhoe in the flatdeck.The rearing results are 2,3 litters per sow and year with losses ca. 1,8 piglets.Every sow per year has a average of 24 piglets.The fatteners from 35 to 100 kg needs 100 - 131 days,as there are growing differences in hot summer and in winter time.
Conclusion: The Xin Xing° farm organisation in Liuzhou city,Guang Xi province PR China has build up a SPF farm with a "three-side-production" for selling breeding pigs and fatteners. To the farm belongs a semen station,a feed mill and a laboratory for testing blood samples from the own pigs. As the health status is high only selling pigs for breeding are vaccinated for PV2 and PRRSV.The own pigs are controlled in the owns laboratory by PCR for diseases.
Keywords:SPF,three-side-production

Disclosure of Interest: None Declared

Keywords: None

Herd Health Management and Economy

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The economic impact of an outbreak of Mycoplasma hyopneumoniae

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Introduction: The Porcine Respiratory Disease Complex (PRDC) is a common problem in modern pig production worldwide and involves infections by different viral and bacterial species, and is also influenced by management and housing. To give good advice about how to cope with these infections, it is necessary to know at what age the pigs are infected. The ResPig-program (supported by MSD) is a good tool for this as it is based on a combination of blood-samples from a cross-section of the animals and postmortem examinations. The aim of this study was to identify the cause of coughing in a farrow-to-finish herd, to introduce an intervention program and follow up on the economic effects.
Materials and Methods: The farm had 700 sows with frequent coughing among fatteners with a high lung score registered at slaughter (≈35 % pleuritic and ≈ 20% pneumonia). Blood samples were collected from 36 pigs in 6 different age groups: 6, 10, 13, 17 and 22 weeks of age and gilts at 26 weeks of age. The blood samples were analyzed for antibodies against APX I, APX II, APX III, Influenza, *M hyo, App* serotype 2 and 3, *P. multocida* and *PCV.* Postmortem examinations were performed in 3 pigs. After analysis of results combined with the clinical symptoms, a mycoplasma (ThoroVax, known as M+Pac in other countries, MSD Animal Health) vaccination program was initiated: 7 week old pigs were vaccinated and gilts were boosted before mating. Data on rearing period, weight and remarks at slaughter was gathered.
Results: No *Influenza* or *P. multocida* were detected in the samples and there were only low titers against *PCV2.* Gilts had a positive titer for *M hyo* at 26 weeks of age. In one pig (22 weeks old), *M hyo* was detected by PCR and there were signs of EP. APX I-III antibody titers were high in all groups no matter the age. The APP 2 test showed antibody reactivity in pigs older than 10 weeks. One year after the initiating of the vaccination program coughing had vanished. The incidence of EP registered at slaughter was reduced from 21.5 to 1.3 % and pleuritis from 33.5 to 15 %, respectively. The daily weight gain had increased with almost 100 gram and reduced the rearing period with 10 days. The vaccination program had an ROI of 2, 6 € with a 0, 86 € investment (including cost for vaccine and work).
Conclusion: PRDC is a common and costly problem for Swedish pig producers. Each pig with pleuritis at the abattoir has 2, 15 € deducted on top of the reduced growth and feed efficiency. The ResPig-program combined with economic analysis of production is a useful tool to assist in diagnosis of PRDC and decisions about intervention programs.

Disclosure of Interest: None Declared

Keywords: Economics, Mycoplasma hyopneumoniae

Herd Health Management and Economy

PO-PT2-294

Reticulocytes response by flow cytometer and Thiazole Orange staining of newborn piglets with iron deficiency anemia (IDA)

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Introduction: Originate in the bone marrow from the orthchromatic erythroblast by nuclear, the reticulocytes (RETs) are immature red blood cells. Into peripheral blood, the RETs counts are the diagnostic indicator of erythropoietic activity because through them we obtain information about the functional integrity of the bone marrow, helpful for evaluation, classification and response to therapy of anemia. The flow cytometry (FACS) and Thiazole Orange has the superior method over manual counting (New Blue Methylene), because is faster than microscopic counting, more reproducible and less error. Towards this technique, our goals was to follow the reticulocytes response by Thiazole staining with flow cytometry of newborn piglets were kept on concrete and not supplemented with dextran iron.
Materials and Methods: Collect blood aseptically by veninpuncture of jugular vein into tube sterile K_2 EDTA blood collection tube at five times (3, 7, 14, 21 and 28 days of age). For analyzer red blood cell parameters (RBC, Hemoglobin and PCV) used the cell blood ABCVet counter. The RETs counts (%) used the Thiazole method by commercial reagent Retic-COUNT and data acquisition and analysis are performed with flow cytometry BD FACSCantoII with a 488-nm (FL1) laser, set forward scatter (FSC) e side scatter (SSC) amplifier gains to log mode, low flow, acquire up to 50,000 events, zero compensation for FL1 and gate around only red blood cell population. All results expressed in median and were compared with reference hematology values Thorn (2012) established of piglets 0 – 36 days of age husbandry on concrete floor. For data analysis of acquired samples and for statistical analysis used the software BDFACSDiva and SigmaPlot, respectively.
Results: The piglets at 3 days of age (Day 3) only showed a decrease in the levels of hematocrit (25,4 %). They had anemia at Day 7, 21 and 28 with diminished values of hemoglobin and hematocrit. However at Day 14, the newborns had increased of erythrocyte levels slightly above de reference value and statistical different other days. The RETs counts were: Day 3 (0.3%), Day 7 (5.45%), Day 14 (9.15%), Day 21 (7.5%) e Day 28 (5.7%), only Day 14 there was not reticulocytopenia. Although RETs counts to increase over the days, there was always a marked reticulocytopenia assosciated anemic state. The iron deficiency anemia (IDA) is the more prevalent cause of anemia in newborn piglets. The literature report in IDA, the bone marrow response with reticulocytosis (Day 14), if the privation continues, there is not resolution of anemic state (dyserythropoiesis).
Conclusion: With FACS and Thiazole was possible to evaluate the reticulocyte response of piglets concurrently in iron deficiency anemia.

Disclosure of Interest: None Declared

Keywords: flow cytometry, piglets, reticulocytes response

Poster Abstracts

Miscellaneous

PO-PC03-002

Characteristics of newspaper and google searched articles about porcine epidemic diarrhea compared to dengue fever

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Introduction: The first outbreak of porcine epidemic diarrhea (PED) since 1996 in Japan was reported in October 2013. Also, in August 2014, Japan also had the first outbreak of dengue fever (DF) since 1945. A concern with these recent incidents is the risk of a decrease in pork consumption due to misinformation or exaggeration by the news media. Therefore, our objectives were to characterize PED and DF newspaper and google-searched articles, and identify important text in the articles.
Materials and Methods: Relevant news articles during 53 weeks from January 5, 2014 to January 3, 2015 were identified by using “PED” or “DF” as the search word, by either a google search or downloaded from the Asahi news article database (Kikuzo, Asahi Shinbun, Tokyo, Japan), which is owned by the second largest newspaper company in Japan. The search period did not start from 2013 because DF had not yet occurred and the number of news articles about PED was still very low prior to 2014. The Asahi database was chosen because of its easy-access and extensive news coverage. All analyses were performed in Statistix 9 (Analytical Software, Tallhassee, FL).
Results: The google search identified about 50 times more articles about DF (35,570) than those on PED (644). The DF articles steadily increased after January and peaked in summer, when there were 2,000 or more articles per week for 11 weeks. Furthermore, there were additional peaks 5 weeks and 11 weeks, after the first peak ended. In contrast, the number of googled articles on PED peaked in March when there were 60 or more articles per week for 3 weeks. Again there was a later peak, 11 weeks after the first peak ended.
There were 322 newspaper articles about PED over the 53 weeks compared with only 215 DF articles. However, the number of words per PED article (313 words) were less than in the DF articles (688 words).
Of the 332 PED newspaper articles, 13.5% included a phrase like “it is not infectious to humans”, 3.2% had a phrase meaning “PED pork is still edible” and 4.7% contained a phrase saying “PED pigs can be sold at market.” Additionally, 5.4% of PED articles were on the local news pages, whereas 56.3% of the 215 DF articles were on the national news pages.
Conclusion: Articles about PED were not as sensational as those about DF. The internet information appears to be more sensationalized than that in newspapers, although there was less information in the newspaper articles stating that “the pork is still safe to eat.” Therefore, this study shows that the swine industry needs to, disseminate more information on pork safety when diseases occur.

Disclosure of Interest: None Declared

Keywords: dengue fever, media, porcine epidemic diarrhea

Miscellaneous

PO-PC03-016

Effect of an Anti-GnRF vaccine (Improvac®) on animal performance and cutting yields of light weight male finishing pigs

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Introduction: The potential benefits of using an Anti-GnRF vaccine (Improvac) rather than physical castration on animal performance and primal cut yields were assessed in light weight pigs.
Materials and Methods: The field trial used a complete randomized block design and 801 male pigs were allotted to one of two treatments at birth: Barrows (B, n=398) and Improvac males (IM, n=403). Improvac was administered to IM at 90 and 120 days (d) of age. Pigs were slaughtered at 154d of age. Initial (IW) and final individual weights (FW) were recorded at 70 and 154d of age, respectively, and overall average daily gain (ADG) was calculated. Feed intake was recorded throughout the finishing period and average daily feed intake was calculated (ADFI). Feed conversion rate (FCR) was calculated from ADFI and ADG. The diet fed was identical for both groups and formulated to meet the requirements of B. After slaughter, 32 carcasses at target weight of 76 kg were selected (16 per treatment) for the cutout evaluation. Primary cuts were weighed and primary cut yields (PCY) expressed as percentage of the hot carcass weight. Evaluated primary cuts were: bone-out shoulder, bone-out collar, bone-in loin, ribs, tenderloin and bone-out ham. Also, lard and fat-on skin were analyzed as fat sub-products indicators. Data were analyzed using Mixed Procedures of SAS to perform an ANOVA.
Results: Overall, IW was heavier (P<0.0001) for B (28.38kg) compared to IM (26.99). At the end of the trial, FW was heavier (P<0.03) for B (106.55kg) compared to IM (104.70). Regarding overall animal performance, ADG (70—154d) was not different (P>0.74) between IM (0.898kg) and B (0.902). However, ADFI was decreased (P<0.01) for IM (2.089kg) compared to B (2.267). As a result, the FCR was decreased (P<0.007) for IM (2.33) compared to B (2.51). Carcass target wt. was not different (P>0.94; IM= 76.53 vs B= 76.47kg). However, PCY was increased (P<0.03) for IM (76.1%) compared to B (74.3%). Bone-out collar was heavier (P<0.02) for IM (7.86%) compared to B (7.43%). Some primary cuts tend to be heavier in IM carcasses; ribs (P>0.09) and tenderloin (P>0.06) were heavier in IM (13.7% and 2.0) compared to B (13.4% and 1.9). No statistical differences were detected for ham (P>0.17), shoulder (P>0.22) and bone-in loin (P>0.33). Finally, fat indicators such as fat-on skin (P<0.02) and lard (P<0.06) were decreased for IM (22.3% and 1.1) compared to B (23.8% and 1.3).
Conclusion: The known benefits of an Anti-GnRF vaccine in improving feed efficiency and primary cut yields were confirmed in the specific market segment of light weight slaughter, despite non-optimized nutrition for IM pigs.

Disclosure of Interest: D. Fernandez-Dueñas Conflict with: Zoetis Employee, S. Gómez Conflict with: Zoetis Employee, A. Aldaz Conflict with: Zoetis Employee, J. Allison Conflict with: Zoetis Employee

Keywords: Anti-GnRF vaccine (Improvac), Light weight, Male finishing pigs

Miscellaneous

PO-PC03-017

Monitoring of AMR (Antimicrobial Resistance) in a large pig production system in Italy

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Introduction: Extended-spectrum penicillins have a similar spectrum of activity to 2nd and 3rd generation cephalosporins and they include aminopenicillins such as amoxicillin and ampicillin and amoxicillin in combination with clavulanic acid.Bacterial resistance to extended-spectrum penicillins has evolved rapidly in recent years specially in *Enterobacteriaceae* because these bacteria could carry beta-lactamases capable of hydrolyzing important beta-lactam antimicrobials (EMA, 2015). It has also been assessed by the EMA in 2015 that the veterinary use of aminopenicillins might have the ability to facilitate the spread of antimicrobial resistance similarly to 3rd and 4th-generation cephalosporins.
Materials and Methods: In order to monitor a possible change of sensitivity in time of amoxicillin, ampicillin, amoxicillin-clavulanic acid and ceftiofur (3rd-generation cephalosporin) in a large production system in Italy during years 2011-2015, we gathered data from the automatic Disc Diffusion susceptibility tests micro-reader SIRSCAN Micro ™ (I2A, France) database. These data were obtained from swine bacterial strains isolated from field clinical cases and namely enteric pathogens belonging to the *Enterobacteriaceae* family (haemolytic *Escherichia coli – HEC*), respiratory pathogens (*Pasteurella multocida – PM*) and *Streptococcus suis* (*SS*). AMR was determined using the Kirby-Bauer disk diffusion technique on Mueller-Hinton agar (OXOID, UK) following CLSI guidelines.
Results: From 2011 to 2015, 1714 bacterial strains were tested for AMR (731 *HEC*, 226 *PM* and 757 *SS*). *HEC* maintained a constant and high level of susceptibility to ceftiofur ranging from 73,46% in 2015 to 86,23% in 2013 with an average of the 78.06%. In contrast, the lack of efficacy for amoxicillin and ampicillin was documented by a lower number of susceptible strains (9,50% and 9,44 respectively). Amoxicillin and clavulanic acid susceptibility was also less than ceftiofur and ranged from 34,56% in 2013 but increasing up to 54,79% in 2015. *PM* and *SS* showed more than 90% of susceptibility to all antimicrobials tested but ceftiofur that, for the latter, reached an average of 75,57% in the 5-year period.
Conclusion: In spite of a rather common usage of aminopenicillins and their combination with beta-lactamase inhibitors, in the 5-year period 2011-2015, *SS* and *PM* are still highly susceptible to these antibiotics and to 3rd generation cephalosporins as ceftiofur. On the other hand, in the same period of time, *HEC*, causative agent of the swine post-weaning diarrhea, reveals resistance to these first substances but still maintaining a high level of susceptibility to ceftiofur without an increase of level of resistance.

Disclosure of Interest: None Declared

Keywords: aminopenicillins, AMR, Cephalosporins

Miscellaneous

PO-PC03-019

Evaluation of female pigs injected with Improvest and raised in mixed gender pens or gilt only pens in Canadian commercial production systems

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Introduction: Managing finishing pigs all-in/all-out is important to maintain health status. Depending on herd size this may be easier to accomplish when genders are mixed. In the context of heavier & older market pigs, temporary suppression of estrus in gilts reared with males may bring production benefits; such as reduced unwanted behaviours and increased time feeding, potentially minimizing stress and performance differences. This report evaluates hormone levels, slaughter parameters and behaviour in female pigs dosed with Improvest and raised in mixed gender pens or gilt-only pens in Canadian commercial production systems.
Materials and Methods: The use of Improvest in female pigs was evaluated in two trials; the first trial in gilt-only pens raised alongside pens of Improvest males, the second trial in mixed gender pens. In both trials, dedicated study pens also included control female pigs which did not receive Improvest. *Gilt-only* (study1): At entry in finisher, two rooms had one designated study pen each which contained half Control females(CF) and half Improvest females(TF) for a total of 50 animals/study pen. *Mixed gender*(study 2)*:* At entry in finisher, a pool of 40 healthy females were uniquely identified then randomly allocated to receive TF (n=20) or CF (n=20). These females were mixed in two pens with five Improvest males(IM) added to each pen. In both studies, CF and TF were tagged and bled for progesterone and estradiol assessment at 3 time periods: after randomization and assignment to pen; prior to the second dose of Improvest; and just prior to shipping. CF and TF in dedicated study pens were evaluated for behaviour 2 weeks after second injection of Improvest and just prior to slaughter. At slaughter, carcass weight, back fat and loin depth were measured.
Results: In study 1, 1 CF gilt had sera progesterone of 16 ng/ml prior to slaughter while no TF gilts were higher than 5 ng/ml. In study 2, estradiol was significantly ($p<0.05$) lower in TF than in CF (0.95 vs 1.88 pg/ml) just prior to slaughter. In study 1, TF gilts reached 104 kg of carcass weight in 110 days compared to 116 days for CF gilts. In study 2, back fat was significantly greater ($p<0.05$) for TF than for CF (16.9vs13.8mm). Carcass weight was 105 kg for TF and 101kg for CF after 103 days in finisher. For both studies, no signs of estrus were detected at the first observation time. Just prior to slaughter, 3 CF gilts in study 1 and 4 CF gilts in study 2 had red swollen vulvas.
Conclusion: The use of Improvest in female pigs suppresses estrus at the end of the finisher period while allowing them to reach market weight faster without expressing unwanted behaviours.

Disclosure of Interest: C. Surprenant: None Declared, K. Talbot: None Declared, B. Laplante: None Declared, J. Daigneault Conflict with: Employee of Zoetis, L. Van De Weyer Conflict with: Employee of Zoetis

Keywords: gilt, Improvest

Poster Abstracts

Miscellaneous

PO-PC03-020

Cough Control Program for the identification of respiratory pathogens in PRDC farms

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Introduction: In fattening pigs, PRDC is the main factor for reduced performance globally. Main pathogens for PRDC are *Mycoplasma hyopneumoniae* (M hyo), Porcine Reproductive and Respiratory Syndrome virus (PRRSv), Porcine Circovirus type 2 (PCV2) and Swine Influenza Virus (SIV). Especially in farms vaccinated for some or all of these pathogens, the evaluation of any responsible pathogen for respiratory problems is difficult. In countries where farmers do not that easily agree to diagnostic necropsies for proper investigation, the efficacy of M hyo vaccines is quite often in doubt. In Denmark, a program called “three-in-one diagnostics” revealed promising results with regard to a first insight of possible pathogens responsible for the respiratory problems. The objective of this investigation was the evaluation of European farms with a slightly modified program.
Materials and Methods: In total, 25 farms were investigated from which 8 served as controls (no relevant respiratory symptoms) and 17 as case farms where cough was observed in pigs at the age of 5 to 20 weeks. The program consisted of collecting 9 Oral fluid (OF) samples with 3 ropes each in the group of coughing pigs, pigs about 3 weeks younger and a group about 3 weeks older, respectively. OFs were investigated by PCR for SIV, M hyo, PRRSv and PCV2. In addition 20 blood samples for serology of the oldest fattening pigs in the farm were investigated for M hyo and in case of inconclusive PCR results additionally for SIV, PRRSv or APP. Cough index of the coughing group was evaluated according to the method described by Nathues et al. A rate of M hyo seropositive samples of 50% or lower in the end of fattening was rated to be an indication that M hyo is not a relevant pathogen in this farm.
Results: In 13 of the 17 case farms (76%) M hyo could be excluded due to the low serological prevalence and/or very low detection rate ahead, at and after cough as well as detection of other pathogens. In these 13 farms cough was most likely due to PRRSv or SIV and in 3 of these farms the reason was unclear.
In 3 farms (18%) an M hyo involvement was obvious but other pathogens might as well be the primary pathogen for cough and in only 1 farm (6%) M hyo was probably the dominating pathogen.
Conclusion: The systematic evaluation provides indications with regard to the relevant pathogen which allows better conclusions. In the majority of investigated farms, cough was not related to M hyo even though it was in most cases highly suspected. However, in some farms further diagnostics are required, preferably full post mortems, especially when a 100% reliable diagnosis is required. It has to be considered that the program provides just a snapshot in time.

Disclosure of Interest: None Declared

Keywords: Diagnostics, Oral fluids, respiratory disease

Miscellaneous

PO-PF3-305

Neurolymphomatosis in a fattening pig with multicentric T-cell lymphoma

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Introduction: Lymphomas in domestic animals usually arise from either B-cells or T-cells, which can be determined by immunohistochemistry. An uncommon manifestation primarily of B-cell lymphoma is called neurolymphomatosis, represented by a neoplastic invasion of cranial nerves and peripheral nerve roots, plexus, or nerves. Peripheral nerve involvement of malignant lymphomas in domestic mammals is rare and only single cases have been described in dogs, cats, and horses.
Materials and Methods: This case report describes an approximately four months old crossbred castrated male pig (45 kg BW) with diagnosed ulna osteochondrosis by X-rays, fever (41.2ºC) and mild diarrhea. Antibiotic and antiphlogistic treatment for six days turned in a temporary recovery. Two days after the last treatment, the body temperature increased again (41.6ºC). The pig showed progressive listlessness and anorexia, hemorrhagic diarrhea and recumbency. Hematologic analysis revealed a marked leukocytosis with 84% blast cells, showing a scant, agranular cytoplasm and prominent nucleoli, indicative of lymphoid leucosis. The animal was euthanized and the carcass was investigated.
Results: At postmortem examination a severe generalized lymphadenopathy with enlarged lymph nodes up to 6 cm in diameter with mottled, partly nodular cut surface could be seen. Additionally, a massive hepatomegaly and splenomegalie were observed. The kidneys showed multifocal to coalescing white areas in the cortex and medulla. In the abdominal cavity 600 ml of a turbid serous fluid with fibrin was found.
Histology revealed the presence of monomorphic neoplastic round cell infiltrates in the lymph node, bone marrow, tonsils, lung spleen, liver, kidney, urinary bladder, and intestine as well as in cerebral and cerebral meninges. In addition, peri- and endoneural round cell invasions were found within the sciatic and medial plantar nerve of the left hind leg. Using immunohistochemistry the majority of neoplastic round cells express CD3, while no expression of CD79a and lysozyme was observed within the tumor, characteristic of T-cell origin.
Conclusion: Multicentric lymphomas are rather common neoplasms in pigs, which usually affect lymph nodes and different organg systems. An uncommon manifestation of malignant lymphoma represents neoplastic nerve involvement. This case is the first report of neurolymphomatosis in a pig with multicentric T-cell lymphoma. The pig did not show any signs of neurological deficits, which is in accordance to clinical observations in man, and leads to the assumption that the involvement of nerves in multicentric lymphomas remains frequently undiscovered.

Disclosure of Interest: None Declared

Keywords: leukemia, peripheral nerves, T-cell lymphoma

Miscellaneous

PO-PT2-004

Effect of an Anti-GnRF vaccine (Improvac®) in ractopamine-free male and female pigs, part I: weight gain and carcass characteristics

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Introduction: The study assessed the growth and carcass characteristics of finishing pigs vaccinated against GnRF (using Improvac®) and intended for ractopamine-free export markets. In Mexico, Improvac is approved for oestrus suppression in gilts and boar taint control in males.
Materials and Methods: The field trial used 780 commercial pigs (380 females, 400 males) in a complete randomized block design. Pigs were allotted to one of four treatments at birth: Gilts (G), Improvac gilts (IG), Barrows (B) and Improvac males (IM). From weaning, pigs were housed in pens of approximately 25 (2 pens per treatment for 4 blocks). Improvac was given at 70 and 134 days (d) of age to IG and IM. Diets were formulated to meet the nutritional requirements of non-Improvac pigs with no inclusion of ractopamine. All pigs were individually weighed and tagged at the beginning (IW = 50d) and the end of the trial (FW= 180d) and average daily weight gain (ADG) calculated. After slaughter, prior to chilling, hot carcass weight (HCW), back-fat depth (BFD) and muscle depth (MD) were recorded automatically at P_2 (approximately 6.5 cm off middle-line). Cold carcass weight (CCW) was recorded 24h after slaughter. Data were analyzed using Mixed Procedures of SAS.
Results: IW was different among treatments (P>0.02), where IG (17.26kg) were heavier than G (16.50) and IM (16.77), but similar to B (17.01kg); B were similar to IM. For FW, B (124.81kg) and IM (122.11 kg) were not different; but both treatments were heavier (P<0.0001) than IG (117.51), and IG were heavier than G (108.58kg). ADG was greater (P<0.001) for B (0.828kg) and IM (0.810) compared to IG (0.771), and IG was greater than G (0.710kg). Regarding HCW, B (101.26kg) were heavier (P<0.0001) than G (87.73), IG (94.53) and IM (96.22kg); however, IG and IM were not different. For CCW, B (99.95kg) carcasses were heavier (P<0.0001) than G (86.57), IG (93.32) and IM (95.25kg); however, IG and IM were not different. Regarding carcass characteristics, BFD for B (18.55mm) and IM (18.55) were greater (P<0.0001) than G (13.73) and IG (17.06mm); BFD for IG was greater than G. Finally, in terms of MD, B (63.52 mm) was similar to IG (62.77) but deeper (P<0.006) than IM (60.82) and G (61.73mm); IM was similar to G but different to IG; G and IG were similar.
Conclusion: As a result of oestrus suppression (2^{nd} dose 6.5 weeks prior slaughter), gilts were heavier at the target market age and carcasses were fatter, probably due to an increased feed intake. These characteristics could represent an opportunity to homogenize the carcass weights and characteristics of mixed sex populations intended for export markets.

Disclosure of Interest: D. Fernández-Dueñas Conflict with: Zoetis employee, J. Morales Conflict with: DSM Employee, R. Magaña Conflict with: DSM Employee, J. Gabriel Conflict with: DSM Employee, A. Aldaz Conflict with: Zoetis employee, J. Allison Conflict with: Zoetis employee

Keywords: Anti-GnRF vaccine (Improvac), Female pigs, Ractopamine-free

Miscellaneous

PO-PT2-005

Auditing a cleaning and disinfection trailer-transportation protocol with AccuPoint Advanced®

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Introduction: Bio-load reduction on surfaces is critical to improve the efficacy and efficiency of disinfectants and other products that could be affected by organic matter and prior biofilm development.
When asked to veterinarians; how the effectiveness of these routine processes are evaluated, answers vary. Some use surface sampling with culture plates and/or PCR to determine the presence of bacterial and/or virus contamination. However, even when these processes are used, they are only used periodically. And, there are no monitoring programs available. This proves to be a concern as livestock transportation could be the initial sites for early bio-load accumulations that may lead into early contaminations.
Materials and Methods: Trailer transportation plays a critical role in the dissemination of Porcine Epidemic Diarrhea virus (PEDv) and other swine pathogens. Trailers from a swine transportation unit were randomly tested using AccuPoint Advance.
AccuPoint Advanced ATP Sanitation Monitoring system: is based on the detection of adenosine triphosphate (ATP) The AccuPoint system consists of a reader that detects the presence of ATP on surfaces through the production of light when ATP is combined with two enzymes in the system's samplers, luciferin and luciferase. The AccuPoint reader calculates the light produced in relative light units (RLU), and the sample's RLU results are directly correlated with the level of cleanliness of the sampled surface — the higher the RLU result, the more ATP that was detected on the surface
Results: ATP results were categorized as pass (green) at less than 150 RLU, marginal (yellow) between 150-300 RLU, and fail (red) when results were greater than 300 RLU.
This technology can be used by any animal production unit to establish their baselines to determine more objective and accurate audits. These results can be obtained in less than 20 seconds and the sampling can be performed right after the surface dries.
AccuPoint Advanced can help establish permissible ATP levels for each type of surface and to establish allowable ranges (cleaning and disinfection indexes). It can also perform evaluations in a more objective way, and generate specific data to transportation units
Conclusion: Values over 400 RLU were associated with unclean surfaces — where adjustments to hygiene protocols should be made in order to achieve RLU values lower than 300. These results may vary from company to company, a baseline could be in the range from 300 to 400 on acceptable levels for a cleaning and disinfection protocol (See Graph 1), but AccuPoint Advanced is able to show activity between 0 to 99.999 to allow broader and customize baselines; according with the bio-load tolerance for each condition

Disclosure of Interest: None Declared

Keywords: ATP, Disinfection, Transportation

Poster Abstracts

Miscellaneous

PO-PT2-006

Production performance of gilts following estrus suppression (using Improvac®) in the grow-finish period.

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Introduction: Improvac® (Zoetis Inc.) is a global immunological product or vaccine for the control of boar taint in pork from male pigs. In several countries it is also indicated for temporary estrus suppression of gilts. Females usually grow and perform at poorer rates than males, resulting in less productivity. While gender itself is a factor impacting pig performance, sexually maturing gilts may reduce their feed intake resulting in lower growth rates compared with counterpart males. The objective of this study was to compare the performance of normally maturing *vs.* estrus suppressed gilts during growing-finishing production phase.
Materials and Methods: One hundred eighty six healthy 96 day old gilts in a commercial finisher farm in Ecuador were selected and randomly allocated to 2 treatment groups: untreated controls - T01 (n=93) or treated with Improvac - T02 (n=93). The 2mL dose of the vaccine was administered subcutaneously twice, at 96 days and at 120 days of age. Gilts in each treatment group were distributed among 3 equal contiguous pens with 31 pigs each, sharing the same airspace. All pigs in the study were individually weighed at start and every week during the duration of the study (10 weeks). Both groups received the same commercial diets, including ractopamine in the last 4 weeks of the study; feed consumption was recorded daily. All animals in the study were slaughtered at 166 days of age, i.e. 6.5 weeks after the second dose of Improvac.
Results: Both groups had similar starting average weights, 44.39 kg (SD=5.24) for T01 and 44.92 kg (SD=4.92) for T02 (NS). There was no mortality in any of the groups. At the time of slaughter, the Improvac gilts were 10.98 kg heavier ($P \leq 0.01$) than the intact females with the same age; final average weights were 120.76 kg (SD= 8.57) and 109.78 (SD=10.75), respectively. For Improvac gilts the feed conversion rate was lower (2.27 *vs* 2.49), the average daily intake higher (2.14 *vs* 2.04 kg) and the average daily weight gain also higher (1083 *vs* 934 g; $P \leq 0.01$) than the controls. The economic analysis for the use of Improvac resulted in a return on investment of 3.64.
Conclusion: Improvac managed gilts performed better than cycling gilts as they increased significantly the feed consumption after the second dose, resulting in faster growth and heavier slaughter weights. Improvac may help producers to improve the production performance of females in the latest stages of finishing by suppressing sexual activity and the associated behavior. The vaccination protocol can be customized to the specific production objectives of a particular farm, and if used in both males and females, there would be no need to separate sexes in finishing pens.

Disclosure of Interest: K. A. Castillo-Sagbay Conflict with: research grant from Zoetis, J. G. Pérez-Villacís Conflict with: research grant from Zoetis, J. A. Mosquera-Andrade Conflict with: research grant from Zoetis, J. F. Estrada-Pineda Conflict with: Zoetis employee, A. Aldaz Conflict with: Zoetis employee

Keywords: estrus suppression, gilts, Improvac

Miscellaneous

PO-PT2-011

Gene expression profiles of sow's T cells are characteristically distinct in colostrum compared with her own blood

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Introduction: Piglets can only acquire passive immune from colostrum because epitheliochorial nature of porcine placenta does not allow transfer of immune materials from sow to fetus. Colostrum contains not only immunoglobulins but also mononuclear cells, and the colostral cells can be transferred into newborn piglets, but sow's blood cells can't (Tuboly et al., 1988; Williams, 1993). In addition, it is suggested that antigen specific T cells induced by vaccination to sows may transfer to newborns via the ingestion of colostrum (Bandrick et al., 2008). These facts suggest that colostral T cells may have some structural and/or functional characteristics in comparison to blood T cells, but there are a few studies that investigated that difference. Hence, the aim of this study was to reveal and compare the gene expression profile in cytotoxic T cells (CTL; $CD8^+$) and CD4/CD8 double positive T cells (DPTL) which are major T cell subsets in porcine colostrum in each colostral and blood lymphocytes.
Materials and Methods: Colostrum and blood was collected from two sows (Landrace x Large white) and lymphocytes were isolated. CTL and DPTL were fluorescently labeled with anti CD3, CD4 and CD8 antibodies and each T cell subset was separately sorted by a cell sorter. Total RNA was extracted from the sorted cells and gene expression profiles were analyzed by using Porcine oligo DNA microarray (Agilent Technologies). Then, the differentially expressed genes between colostral and blood T cells were selected. Finally, colostrum and peripheral blood samples from eight sows including two sows as described above were used for qRT-PCR analysis to validate the noted genes *FOS*, *NFKB1*, *IFNG*, *CXCR6*, *CCR5*, *ITGB2*, *CCR7*, and *SELL*.
Results: Gene expression profiles indicated that, unlike in blood, numerous cell signaling pathways might be activated in colostrum. Profile analysis also showed that transcription factors *FOS* and *NFKB*1 were involved in most cell signaling pathways, and that the expression of these factors was significantly higher in colostral T cells than in blood T cells ($P < 0.05$). Furthermore, T cell differentiation markers *CCR7* and *SELL* in colostral T cells showed expression patterns closely similar to those found in effector and/or effector memory T cells (Broere et al., 2011).
Conclusion: In conclusion, either all or predominant T cells in colostrum display an effector-like phenotype and thus are more activated than those in blood. This gene expression profile enables T cells to migrate to mammary glands and be secreted in colostrum. Eventually, circulating colostral T cells are likely to cause development of the immune system in newborn piglets.

Disclosure of Interest: None Declared

Keywords: Corostral and peripheral blood T cells, DNA microarray, qRT-PCR

Miscellaneous

PO-PT2-012

Learnings from on-site anemia measurements with HemoCue 201 in piglets around weaning

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Introduction: Newborn piglets are at risk for developing iron deficiency because they are born with limited iron stores and sow milk provides them with only ±1 mg of iron per day. Iron deficiency leads to anemia characterized by a low concentration of iron-containing hemoglobulin (Hb) in red blood cells. Therefore, iron administration to piglets within the first week of life is common practice in pig farms. The aim of this study was to investigate the effect of iron supplementation method (1) and age at sampling (2) on the Hb concentration in piglets at weaning.
Materials and Methods: Seventeen sow herds were included. Piglets were weaned at either 3 weeks (3W, n = 14 farms) or 4 weeks of age (4W, n = 3 farms). From the farms weaning at 3W, nine treated piglets via intramuscular injection of 200 mg iron (3W-IM) and five supplemented iron via the drinking water (3W-PO). Farms weaning at 4W treated the piglets with 200 mg iron via injection (4W-IM). Two farms (3W-IM and 3W-PO) reported clinical signs of anemia in suckling piglets. At each farm, 20 piglets belonging to one farrowing group were selected randomly at weaning ±3 days. The Hb concentration of each piglet was measured on-site using a HemoCue 201+ analyzer (HemoCue® Diagnostics B.V., The Netherlands). Data were analyzed via the Kruskal-Wallis test because Hb concentrations after oral supplementation were not normally distributed.
Results: Based on all data (n = 340 piglets), 27% of piglets showed anemia defined as Hb concentration <9 g/dL. The highest percentage of anemic piglets was found on farms with oral supplementation (52%). The average Hb concentration and 95% confidence interval were 9.9 [9.7-10.1] (3W-IM), 8.7 [8.2-9.2] (3W-PO) and 10.1 [9.8-10.4] (4W-IM). The Hb concentration was lower in the 3W-PO group compared to the 3W-IM and 4W-IM groups ($p<0.01$). No difference was observed between 3W-IM and 4W-IM ($p=0.54$).
Conclusion: Our study reveals that anemia in suckling piglets is still quite common. The Hb concentration at weaning was significantly affected by the method of iron supplementation. Oral supplementation resulted in a lower average Hb concentration and a higher variation between piglets compared to injection. This is possibly due to variable water intake by suckling piglets. We hypothesized a lower Hb concentration at 4W compared to 3W but this was not confirmed. The piglets included in this study were fed ad libitum with creep feed. Next, we want to investigate whether the uptake of creep feed (as a source of iron) has a positive effect on the Hb concentration in 4-week old piglets. The HemoCue was shown to be a useful tool for on-site monitoring of iron deficiency anemia.

Disclosure of Interest: None Declared

Keywords: Anemia, Hemoglobin, Iron

Miscellaneous

PO-PT2-013

Effects of Improvac® administration to gilts on estrus occurrence, carcass quality and size of reproductive organs.

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Introduction: Improvac® (Zoetis Inc.) is a global immunological product or vaccine for the control of boar taint in pork from male pigs. In several countries it is also indicated for temporary estrus suppression of gilts. While Improvac is widely used in the production of male pigs, and extensively documented in the scientific literature, the use of this vaccine in female pigs has had much less attention. The objective of this study was to assess the effects of estrus suppression with Improvac on heat detection, carcass quality and the size of reproductive organs at slaughter in commercial finishing females, comparing immunized *vs.* non-immunized gilts.
Materials and Methods: One hundred eighty six healthy 96 day old gilts in a commercial farm in Ecuador were selected and randomly allocated to 2 treatment groups: untreated control - T01 (n=93) or treated with Improvac - T02 (n=93). Two doses of 2mL were administered subcutaneously twice at 96 and at 120 days of age, and all animals distributed among 6 equal contiguous pens of 31 pigs each, sharing the same airspace. From day 120 (2^{nd} dose) until day 166 of age, every gilt was individually assessed twice a day for estrus detection. Both treatment groups received the same commercial diets, including ractopamine in the last 4 wks of the study. All pigs were individually weighted on farm and slaughtered at 166 days of age, i.e. 6.5 weeks after the 2^{nd} dose of Improvac. In each treatment group, the 10 gilts closer to the median of final weight were selected for measurements at slaughter: back-fat thickness in P2, length and width of right and left ovaries, uterine horns, uterine body and cervix (mm).
Results: Only one gilt (1.1%) in the Improvac group showed signs of heat from 10 days after the second dose (130 days of age) until the end of the study (166 days), in comparison with 69.9% of control gilts. The average back-fat thickness was 16.05 mm for the control group and 15.21 mm for the treated gilts, but the difference was not statistically significant. Ovaries and uterine horns of Improvac gilts were significantly smaller than control gilts, while there were only numerical but not significant differences in uterine body and cervix sizes between groups.
Conclusion: Two doses of Improvac are highly efficacious in suppressing estrus in young gilts in commercial settings, as proven by no signs for several weeks. The immunization also resulted in a significantly reduced size of reproductive organs when compared to non-immunized gilts. The suppression of reproductive function and sexual behavior induced by Improvac should result in significant benefits for producers. Additional studies are needed for a better understanding of the implications.

Disclosure of Interest: K. A. Castillo-Sagbay Conflict with: research grant from Zoetis, J. G. Pérez-Villacís Conflict with: research grant from Zoetis, J. A. Mosquera-Andrade Conflict with: research grant from Zoetis, J. F. Estrada-Pineda Conflict with: Zoetis employee, A. Aldaz Conflict with: Zoetis employee

Keywords: estrus suppression, gilts, Improvac

Poster Abstracts

Miscellaneous

PO-PT2-014

Reduction of Pseudorabies Incidence Using Ingelvac® Aujeszky MLV in two Malaysian Herd

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Introduction: Sporadic Aujeszky's disease outbreaks have occurred and reported in different parts of Malaysia in year 1998 despite vaccination[1]. The latest prevalence report shows Aujeszky's disease in Malaysian farm is 46.15%.[2] Most Malaysian farmers are practising PR vaccination in breeding herd only while the grower-finisher are left unvaccinated.
This study shows the efficacy of Ingelvac® Aujeszky MLV in reducing the incidence of pseudorabies virus in 2 farrow-finish herds in Malaysia.
Materials and Methods: This study involves 2 herds located in the most denst pig raising areas in Malaysia.
Herd 1: 300 sows, previously used PRV Inactivated vaccine, started to use Ingelvac® Aujeszky MLV in September , 2014 and period of comparison for 6 months and 12 months
Herd 2: 800 sows, previously used PRV modified live vaccine, started to use Ingelvac® Aujeszky MLV in Nov, 2014 and period of comparison for 6 months and 12 months
Prior to the change in vaccination, the 2 herds have been vaccinating sows at 93 days of gestation with or without piglet vaccination. During the eradication phase, sows were vaccinated 3 times per year and piglet at day 1-3 intranasally using Ingelvac Aujeszky MLV
The percentage of seropositive before and after intervention was evaluated with IDEXX® PRV/ADV gI AB test kit.
Results: After using Ingelvac® Aujeszky MLV, the percentage of animals tested seropositive for pseudorabies gE antibody declined in both herds. For herd 1, from a 36% seropositive in the breeding herd, the prevalence was reduced to 0% in 12 months. The fattening herd was reduced to 0% within only 6 months. For herd 2, the breeding herd positive was also reduced from 60% to only 20% after a year while the fattening herd was completely negative after 6 months.
Conclusion: Ingelvac® Aujeszky MLV drastically reduced the incidence of pseudorabies gE seropositive aniamls in both herds. Intranasal route vaccination using Ingelvac® Aujeszky MLV successfully reduced the percentage of gE sero-positive in grower-finisher in both Malaysian herd.
References:
1. Jasbir Singh,.(1998) Epidemiology of Aujeszky's disease in Pigs in Malaysia.
2. Yong. CK,. (2015) Sero-prevelence Status of Pseudorabies In Malaysia.

Disclosure of Interest: None Declared

Keywords: Incidence, prevalence, Seropositive

Miscellaneous

PO-PT2-015

Investigations on bottle mount vaccinator hygiene in north-west-german pig farms

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Introduction: A lot of different syringes are used in pig farms. Very popular are so called bottle mount vaccinators (BMVs). They are used e.g. for vaccination, antibiotic- or iron-injections. These vaccinators very often are made out of plastic and most of them cannot be disassembled, which makes them hard to clean and disinfect. Inspired by an austrian field case in which 32 piglets out of 42 died from an anaphylactic reaction after vaccination with contaminated vaccinators (Langhoff et al. 2015), the hygiene of BMVs in 10 north-west german pig farms was investigated.
Materials and Methods: The study was performed in 9 farms from 150 to 1600 sows and one finisher herd of 1700. Nucleus herds with very high health status as well as farms with poor health status were included. One to three vaccinators per herd were tested, 18 overall. For the collection of samples a 100cc bottle of physiological saline solution was put on the vaccinator and 5 to 10 ml of the solution was injected into sterile a tube and sent to the microbiological laboratory where it was tested semi quantitative on germ content, APP, HPS, *Pseudomonas aeruginosa,* coliforme germs, yeasts and mould fungi.
Results: The BMVs were in use between 4 and 16 weeks on farm. All except for two were just cleaned with water and not disinfected. 2 vaccinators of one farm were not cleaned at all. 4 of the 18 tested BMVs gave completely negative results . In none of the vaccinators APP or HPS was found. In 11 vaccinators unspecific germ content was found, 7 times medium contents. 2 BMVs had low contents of coliform germs and in two high or medium contents of *Pseudomonas aeruginosa.* 12 of the 18 BMVs were contaminated with yeasts and 8 with mould fungi. In 12 out of 18 vaccinators more than one (up to 4) contaminations were found. There was no correlation between health status or biosecurity level (ext. and int.) and microbiological contamination of BMVs or weeks of usage and microbiological contamination.
Conclusion: Vaccinator hygiene in pig farms in practice is an underestimated feature and can lead to problems after vaccination (Langhoff et al. 2015). In Germany BMVs are delivered together with vaccines by the vaccine companies. Thus farmers use these BMVs for a quite long time (up to 16 weeks in this study). Most of these BMVs can not be disassembled or boiled. Because of that farmers just clean them with water. Compared to the austrian study less contamination in vaccinators was found. Farmers should be educated by their vets about cleaning and disinfection of BMVs. Producers of BMVs should give a cleaning manual along and make them easier to disassemble and boilable.

Disclosure of Interest: None Declared

Keywords: bottle mount vaccinator, hygiene, north-west german pig farms

Miscellaneous

PO-PT2-017

Piglets suffering from IUGR show impaired pre-weaning growth performance

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Introduction: Piglets suffering from intrauterine growth restriction (IUGR) are compromised at birth compared to normal piglets. They have lower birth weight and display a less competitive suckling behavior immediately after birth. Survival rate amongst IUGR piglets are lower than normal piglets but can be improved by management interventions. However, it is unknown how IUGR affects growth performance of suckling piglets. The aim of this study was therefore to investigate daily gain and weaning weights of IUGR piglets compared to normal piglets.
Materials and Methods: All piglets born to 203 sows in a Danish production herd were individually tagged, weighed and given an IUGR-score at birth. The degree of IUGR was assessed from head morphology with piglets characterized as 'normal' (n=2.147), 'light IUGR' (L-IUGR, n=391) or 'severe IUGR' (S-IUGR, n=16). At day 14 and 26 piglets were weighed individually and only piglets that were alive at day 26 were included in this study. Data was analyzed using linear models in SAS.
Results: Normal piglets were heavier at birth than L-IUGR piglets (1.5 ± 0.01 vs. 1.1 ± 0.01 kg; P<0.001) and L-IUGR piglets were heavier than S-IUGR piglets (0.8 ± 0.03; P<0.001). By day 26 normal piglets weighed 7.3 ± 0.03 kg, L-IUGR piglets weighed 6.1 ± 0.07 kg and S-IUGR piglets weighed 5.2 ± 0.22 kg (P=0.161). The growth rate from birth to weaning at day 26 tended to be increased for normal piglets compared to S-IUGR piglets (228 ± 1.22 vs. 170 ± 10.02 g/day; P=0.099), but the birth to weaning growth rate of L-IUGR piglets (199 ± 2.51 g/day) was similar to IUGR (P=0.209) and normal piglets (P=0.114). From day 0 to day 14 the growth rate of normal piglets (210 ± 1.32 g/day) was greater than the growth rate of L-IUGR piglets (176 ± 2.57 g/day; P<0.03), but in this period the growth rate of L-IUGR piglets did not differ from the growth rate of S-IUGR piglets (147 ± 12.72 g/day; P=0.28). From day 14 to day 26 S-IUGR piglets grew numerically slower than normal piglets but there was no statistical difference in growth rates of normal piglets (251 ± 1.74 g/day) compared to L-IUGR (233 ± 3.70 g/day) and S-IUGR piglets (199 ± 18.39 g/day) (P=0.443).
Conclusion: Piglets suffering from IUGR had lower birth weights and reduced growth rates to day 14 than normal piglets. However, growth rate from day 14 to day 26 was similar for all piglets and there was no difference in weaning weights. The results suggest that growth performance of IUGR piglets is impaired, especially in the first part of lactation. Further studies of the growth pattern of IUGR piglets are needed as well as studies to establish if IUGR piglets display catch-up growth in the latter part of lactation.

Disclosure of Interest: None Declared

Keywords: growth performance, growth rate, intra-uterine growth restricted piglets

Miscellaneous

PO-PT2-303

Evaluation of double gleptoferron (Gleptosil®) injection on Hb levels

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Introduction: Piglets are born with limited iron reserve and sow milk is not sufficient supplementation. Constant genetic improvement and intensity of growth is characteristic for modern genetic lines and second injection of iron before weaning is becoming more common in the field practice. Hemoglobin (Hb) measurement has been most widely used method to establish optimum iron status in piglets. The aim of this field study was to investigate the effect of second application of gleptoferron (Gleptosil) on Hb in order to evaluate iron status of piglets.
Materials and Methods: A modern farm in total capacity 1200 sows located in Czech Republic with a high health status, DanBreed genetics was selected. Piglets are weaned at 26 days of age. 32 litters were used for the trial and divided at random into 2 groups (A, B). In group A, all piglets were injected by gleptoferron (Gleptosil) according manufacture's recommendation at day 3 after birth. Group B was additionally injected by second shot of gleptoferron (Gleptosil) in two weeks interval (day 17th). Two randomly selected litters from each group were weighed at 3 days of age and at weaning in order to monitor average weight per piglet. All piglets received a 20 % protein creep feed as from 7 days of age.
Results: In total, 32 litters were included in the study divided into 2 groups (one and two applications). The 13 blood samples per group were randomly collected during the weaning and Hb level was detected by haemoglobin-metr (HemoCue Hb 201). Mean Hb level in group A (one application) was evaluated 110,77 g/L (SD 9,8). Group B was obtained higher Hb mean level 121,3 g/L (SD 8,0). We haven't foundstatistical difference between Gleptosil and Gleptosil 2x (p=0.0738), but results in mean are better in Gleptosil 2x group and also variability was slightly better. ADG in group A was 218g in comparison with group B -216 g.
Conclusion: Nowadays modern genetics are selected for fast growth and during the first week of life piglets double their weight and increase plasma volume by 30%, therefore demand for optimum supplementation of iron is important. Additional injection of gleptoferron (Gleptosil) increased blood Hb level by 10,66 g/L. However we haven't found statistically significant difference in Hb between the groups and in case of ADG, we believe that additional iron injection is beneficial especially for hyperprolific litters, where sows milk (low in iron) is still only constant source, as pre starter consumption is normally relatively variable. Further studies and monitoring of performance during the nursery and finishing period will be needed in order to evaluate beneficial effect of second iron injection.

Disclosure of Interest: None Declared

Keywords: Anemia, Gleptoferron

Poster Abstracts

Miscellaneous

PO-PW1-002

Is it feasible to collect oral fluids from litters of piglets before weaning?

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Introduction: In sows and weaned pigs, oral fluids (OF) are usually sampled with cotton ropes and used for serological analysis or direct identification of pathogens. OF sampling is easy, quick and of low stress for animals, being suitable for group diagnosis since OF samples represent a pool of saliva from different pigs from the same pen. The aim of this study was to investigate if it is feasible to collect OF samples from sucklers, in farrowing pens.
Materials and Methods: The study was conducted in one farrow-to-finish farm located in Brittany. OF samples were collected for each litter with a cotton rope (Ø 0.8 cm). OF sampling was tested at 21 and 28 days of age, with 30 or 45 minutes of rope presentation. OF sampling was considered successful when more than 2 ml of saliva were collected. Each suckler was identified with individual marking number between scapulas. Percentage of piglets within a litter chewing the rope and individual interaction time were obtained from video recordings for 57 piglets from 5 litters at each weaning age. Training of litters the day before sampling was also tested at 21 days of age, by letting a rope for 4 hours in the pen.
Results: At 28 days of age, we were able to collect OF samples within 30 minutes for 82% of the litters with an average of 3.6 ml of saliva per litter (n=17 litters). When the rope was in the farrowing pen for 45 minutes, we were able to collect OF samples from 94% of the litters with an average of 3.95 ml of saliva per litter (n=17 litters). At 21 days of age, OF samples were obtained from 62.5% of the litters (30 minutes access) with an average of 3.1 ml of saliva per litter (n=32 litters). With training the day before, OF samples increased to 100% of the litters (n=14 litters) at 21 days. The percentage of piglets chewing the rope were, on average, 84,4% (from 76,9% to 100%) and 94,6% (from 90% to 100%) at 21 and 28 days of age respectively. The duration of interaction per piglet varied, on average, from 513 seconds (30 to 1190 sec) to 612 seconds (20 to 1560 sec) for 21-day-old and 28-day-old sucklers respectively.
Conclusion: This study showed that collection of OF samples from suckling is feasible by presenting a cotton rope in a farrowing pen for 30 minutes, all the more when piglets have been trained the day before by putting a rope for 4 hours. Moreover, the evaluation of the participation of piglets by video showed that OF samples can be considered as a correct sample of the litter. Collecting OF samples from suckling piglets would be useful to monitor vertical transmission of pathogens from the sow to the litter and for serological analysis, especially for the investigation of colostrum transfer.

Disclosure of Interest: None Declared

Keywords: Diagnosis, Oral fluids, Sucklers

Miscellaneous

PO-PW1-003

Effects of antibiotic treatment during the weaner stage on pig performance and health during finishing

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Introduction: The positive effects of antibiotic treatment on growth and health of pigs during the weaner stage are well known. However the long term 'carry-over' effects are less well known. There is some evidence that in-feed antibiotics during the weaner stage may have a detrimental impact on the development of the immune system and therefore negative consequences for pig health. We studied the effects of an antibiotic treatment during the weaner stage on the performance, health and welfare of pigs during the finishing stage.
Materials and Methods: Six weekly batches of 140 piglets each (840 pigs) were split into two treatments during the weaner stage. Control treatment (CT) had no antibiotics in the diet and the treatment group (AB) had in-feed Sulfa-Trimethoprim during the 9 week weaner stage. The farm was positive to influenza, PRRS, APP and had regular episodes of meningitis and ear and tail biting. No in-feed antibiotics were provided in the finisher stage but pigs were grouped after weighing according to treatment (AB; n=15 pens and CT; n=15 pens) for 10 weeks until slaughter at 105kg. Initial and final body weight (BW) and feed intake were recorded. On the day prior to slaughter, all pigs were weighed and their tail lesion (4 point score) and lameness (3 point score) scores were recorded. During the finishing period all instances of general sickness requiring antibiotic treatment, lameness and mortalities were recorded daily.
Results: Results during weaner stage (see poster P158, ESPHM 2015) showed a higher growth rate (+33 g/day; P=0.033) and a higher feed intake (+60 g/day; P=0.051) for AB pigs but no differences in FCR or mortality. Pigs were moved to finisher stage at 45±5.5 kg and there were no effects of the previous AB treatment on growth (P=0.457), feed intake (P=0.876), tail lesion (P=0.573) or lameness (P=0.519) score prior to slaughter, lameness incidence (P=0.252) or mortality (P=0.194) during the finisher stage. However, there was a higher frequency of antibiotic treatments in AB compared to CT pigs (17 vs 11%; P=0.013) during the finisher stage.
Conclusion: In-feed antibiotic treatment of weaners had no effects on growth, feed intake or mortality during the finisher stage or on tail lesion or lameness scores prior to slaughter. However, antibiotic treatment during the weaner stage could have negative effects on general health of pigs during the finisher stage. Further studies are needed to confirm these findings and to further understand the long term effects of in-feed antibiotics in early production stages.

Disclosure of Interest: None Declared

Keywords: antibiotics, health status, welfare

Miscellaneous

PO-PW1-004

Effects of estrus suppression on performance and carcass quality of gilts.

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Introduction: Improvac® (Zoetis, Florham Park, NJ, USA; marketed as Innosure® in Colombia) is an anti-GnRF vaccine for the immunological castration (IC) of male pigs for the control of boar taint. However, there is very limited information available about the effects of IC on female pigs by temporal suppression of estrus. The objective of this study was to compare performance and carcass quality of immunocastrated *vs.* normally maturing females raised in a commercial farm.
Materials and Methods: 229 crossbred gilts (LW x LD x Pi) with initial average weight of 23.0 kg (SD=3.0) were randomly allocated to one of two treatment groups: T1) Control: 107 gilts in 6 pens received a 2mL subcutaneous injection of a 5% dextrose solution at 17 and 22 weeks of age; T2) IC: 122 gilts in 7 pens received two 2mL subcutaneous injections of Improvac behind the ear at 17 and 22 weeks of age. The 13 pens used were similar, allocating 18 pigs at 1.2 m^2/pig, with water and feed offered *ad libitum*. Both treatment groups received the same diet meeting or exceeding recommended nutritional requirements (NRC, 2012) and included 10ppm ractopamine ClH (Paylean®, Elanco) for the last 4 weeks before slaughter at 28 weeks of age.
Results: IC gilts showed significantly higher Average Daily Feed Intake (ADFI; +8.1%, 173 g; 2.27 *vs* 2.10 kg/day; *P<0.005*), Average Daily Gain (ADG; +5.7%, 53 g; 966 *vs* 913 g/day; *P<0.028*) and final weight (FW; +4.8%, 6.47 kg; 140.8 *vs* 134.3 kg; *P<0.027*) than control gilts. Feed conversion rate for both groups was the same (2.45). IC gilts increased ADFI in the last 5 weeks of the study compared with control gilts (3.29 *vs* 2.64 kg/day; SD: 0.19 and 0.24, respectively), coinciding with the transition to a castrated phase (about one week post second dose of Improvac). Back fat was significantly higher in IC *vs.* control gilts (20.9 *vs* 17.7 mm; *P<0.0001*), but the total lean meat yield was similar for both treatments (62.6 *vs* 63.0 kg/carcass; *P>0.05*).
Conclusion: IC is a new tool that may help to improve productivity and carcass quality of gilts. The temporary castration induced after the second dose led to increased feed consumption, daily growth and final market weight with no negative impact on feed conversion ratio. The substantial increase observed in feed intake after the second dose opens opportunities to optimize the nutritional program according to the production objectives. Depending on the market preferences, the increase in fat deposition can also be desirable for high quality pork products. More studies are needed to better understand the implications for pork producers.

Disclosure of Interest: J. H. Agudelo-Trujillo Conflict with: research supported by Zoetis, P. A. Guzmán-González Conflict with: research supported by Zoetis, J. F. Gómez-Betancur Conflict with: Zoetis employee, A. Aldaz Conflict with: Zoetis employee

Keywords: anti-GnRH, feed intake, gilts

Miscellaneous

PO-PW1-005

Genetic variation in erythroid traits for improved resistance in swine

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Introduction: Clinical-chemical and haematological parameters are essential in evaluating the health status of individuals and herds, but the knowledge about their genetic architecture is sparse, especially in swine. Ruling out the genes and variants contributing to phenotypic variation of such traits might help to select for pigs with a higher general resistance to disease. An overwhelming input into the genomic background of red blood cell traits has been provided during recent years. We have identified genetic effects on chromosomes (SSC) 2 and 3 that provide evidence for variation in erythropoietin (EPO) and the erythropoietin receptor (EPOR) as important candidates for more or less favourable haematological values with regard to the manifestation of anaemia in healthy and diseased pigs.
Materials and Methods: In a first step, genetic loci associated with haematocrit, haemoglobin and red blood cells were mapped as quantitative trait loci (QTL, i.e. chromosomal regions that are associated with the values of erythroid traits in healthy and diseased F2 animals). Two QTL mapped to the regions of EPO and EPOR, and new genetic variation (SNPs) within both genes was identified by differential sequence analysis of pigs with distinct haematological values. These SNPs were used to confirm and improve the QTL. In step three, the SNPs were tested in a set of 150 commercial outbred pigs from a high health herd in the field.
Results: Sequence analysis identified 21 SNPs in EPO and 4 SNPs in EPOR. These SNPs improved marker density and the precision of QTL analysis. Based on this higher informativity, QTL were not only confirmed, but rose significantly in power. One SNP in EPOR exon 7 had major effects. Pigs homozygous for cytosine showed superior erythroid values. This effect was also visible in the cohort of healthy commercial hybrid gilts of step three. Cytosine-homozygous pigs had a higher efficiency in the production of erythrocytes than pigs homozygous for the thymine variant.
Conclusion: The results of the present study provide evidence for clinically relevant variation in the EPOR gene. Pigs with a genetically higher potential for erythropoiesis might be less susceptible during events of infectious haemorrhagic diseases and diseases of the respiratory system, the cardiovascular system or the blood. The development of functional SNPs and markers is of high importance in the view of rising genomic selection programs. Both technics may positively contribute to improved swine health and herd prophylaxis in the future.

Disclosure of Interest: None Declared

Keywords: disease resistance, haematology

Poster Abstracts

Miscellaneous

PO-PW1-006

Pathohistological characterization of pigs from different herds with PRDC

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Introduction: Respiratory diseases provide major concerns regarding production efficiency, animal welfare, antibiotic treatment and consumer protection. Variable combinations of interacting etiological agents and management conditions are source of high complexity and led to the term of the Porcine Respiratory Disease Complex (PRDC). Clinical signs appear late in PRDC and even with cross-pathology and lung checks are not suited to detect all relevant components of the disease. Early detection and pathogen specific attribution of lesions is the classical field of histopathology. The aim of the present study was to give a detailed overview on occurrence and quantity of histological parameters in lungs of pigs with PRDC under field conditions.
Materials and Methods: 58 German hybrid pigs (25-30 kg) were obtained from 29 herds with PRDC for routine diagnostics during herd health service. Pigs were clinically examined and dissected. Lung surface was subdivided into 76 triangles and alterations in each part were scored from 0 (no alteration) to 4 (the whole triangle involved). A detailed histological examination was made from 6 lobes of each lung (except intermediate lobe). Samples were taken from the visually most affected triangles. A score based on the complete range of reasonable histological parameters was developed to quantify degrees of the three main types of pneumonia for all lobes.
Results: Interstitial pneumonia, bronchopneumonia and fibrinous/hemorrhagic pneumonia were detected in 70.3, 18.0 and 13.0% of the lung lobes, respectively, representing different grades, in pure ore mixed form. Macrophages and plasma cells in alveolar lumina, bronchi or bronchioli indicated the lowest degrees of pathological lung lesions. Highest degrees were associated with lymphocytes and macrophages infiltrating alveolar septa and the lamina propria of bronchi and bronchioles and with neutrophils in epithelia and lumina of bronchi and bronchioles. Infiltration of interlobular interstitia with lymphocytes and macrophages was preferentially found in lobes with high aberrations, followed by hyperplasia of the BALT.
Conclusion: Lungs from field cases with PRDC showed overlapping degrees of the three major types of pneumonia which could be scored individually, based on histopathological parameters. Distinct histological findings were suitable to define thresholds in degrees of individual pneumonia forms; they might be useful for diagnosis and prognosis in PRDC field cases. Comparing these pathohistological data with clinical and microbiological findings in a next step should generate a deeper insight into development of PRDC and the interaction and significance of pathogens involved.

Disclosure of Interest: None Declared

Keywords: Diagnostics, Histopathology, PRDC

Miscellaneous

PO-PW1-007

Effect of an Anti-GnRF vaccine (Improvac®) in ractopamine-free male and female pigs, part II: growth performance.

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Introduction: The study assessed the growth performance of finishing pigs vaccinated against GnRF (using Improvac®) and intended for ractopamine-free export markets. In Mexico, Improvac is approved for oestrus suppression in gilts and boar taint control in males.
Materials and Methods: The field trial used 780 commercial pigs (380 females, 400 males) in a complete randomized block design. Pigs were allotted to one of four treatments at birth: Gilts (G), Improvac gilts (IG), Barrows (B) and Improvac males (IM). From weaning, pigs were housed in pens of approximately 25 (2 pens per treatment for 4 blocks). Improvac was given at 70 and 134 days of age (d) to IG and IM. Diets were formulated to meet the nutritional requirements of non-Improvac pigs with no inclusion of ractopamine. Of the 780 pigs, 6 pigs/pen (closest to the pen mean) were selected for individual weekly weighing and average daily gain (ADG) calculation. Feed was manually offered and recorded daily on a pen basis, and average daily feed intake (ADFI) calculated. Feed conversion rate (FCR) was calculated from these ADFI and ADG results. Data were analyzed overall (50 to 180d) and in 2 periods (50 to 134d and 134 to 180d) using Mixed Procedures of SAS.
Results: Initial weights were similar (P>0.78; G=18.44kg, IG= 18.81, B= 19.27, IM= 18.81). Final weights were lighter (P<0.003) for G (111.78kg) than IG (118.29), and both were lighter than B (126.59) and IM (127.89). For the total period (50-180 d) ADFI was increased (P<0.0001) for B (2.350kg) v. IG (2.209) and IM (2.229), with both greater than G (2.000). ADG was increased (P<0.0001) for B (0.811kg) and IM (0.821) v. G (0.700) and IG (0.747). Overall FCR was decreased for IM (2.68) v. G (2.91), IG (2.93) and B (2.89). For the 1st period, ADFI and ADG were increased (P<0.01) for B (2.155kg, 0.844kg) v. G (1.864, 0.754), IG (1.906, 0.716) and IM (1.875, 0.759). FCR for G and B (2.53, 2.53) were similar to IG (2.62) and IM (2.42); but was greater for IG than IM (P<0.04). For the 2nd period, ADFI was different among all treatments (P<0.0001), being greater for IM (2.882kg), than IG (2.765), B (2.705) and G (2.320). ADG was increased (P<0.0001) for IM (1.014kg) v. IG (0.875) and B (0.832); both were greater than G (0.675). FCR was decreased (P<0.02) for IM (2.73) v. G (3.31), IG (3.09) and B (3.26).
Conclusion: After oestrus suppression (2nd dose 6.5 weeks prior slaughter), feed intake increased in vaccinated gilts resulting in increased ADG and heavier market weight. In vaccinated males, the FCR advantage was particularly marked after the effect of Improvac 2nd dose is completed, probably due to the increased weight gain.

Disclosure of Interest: D. Fernandez-Dueñas Conflict with: Zoetis Employee, J. Morales Conflict with: DSM Employee, R. Magaña Conflict with: DSM Employee, J. Gabriel Conflict with: DSM Employee, A. Aldaz Conflict with: Zoetis employee, J. Allison Conflict with: Zoetis employee

Keywords: Anti-GnRF vaccine (Improvac), Female pigs, Ractopamine-free

Miscellaneous

PO-PW1-008

Genetic association between arthritis in piglets and reproduction

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Introduction: Apart from ill-thrift, arthritis in suckling piglets contributes to losses and increased labour and use of antibiotics. The aim of this study was to analyse genetic disposition for developing arthritis or not.
Materials and Methods: During two and a half year, all pigs at the research station at Lövsta, Swedish University of Agricultural Sciences, with 110 sows were studied, i.e. 6,787 liveborn piglets, boars (1 Duroc, **D**; 42 Hampshire, **H**; 11 Landrace, **L**; 47 Yorkshire, **Y**) and, sows (207 **Y**, 12 **Y x L**). Each pig had an identity and an individual record. The occurrence of arthritis was registered from birth to 5 weeks.
Results: In total 415 of the piglets were diagnosed with arthritis (6.1%), where of 86% took place during the first 3 weeks of life.
Y-sows; **523** litters, **305** with no arthritis (*58%*, 11.3±3.5), **125** with one piglet with arthritis (*24%*, 12.7±2.9), **93** with more than one piglet with arthritis (*17%*, 14.6±2.9).
Y x L-sows; **27** litters; **16** with no arthritis (*59%*, 14.3±3.3), 6 with one piglet with arthritis **(***22%*, 14.3±1.6), **5** with more than one piglet with arthritis (*19%*, 15±2.9).
In **Y**-sows a significantly ($P < 0.001$) larger litter size was seen in litters with more than one arthritis.

D-boars; **23** litters, **13** with no arthritis (*57%*, 12.5±3.6), **9** with one piglet with arthritis *39%*, (12.7±3.1), **1** with more than one piglet with arthritis (*4%*, 17.0±0.0).
H-boars; **192** litters, **110** with no arthritis (*57%*, 11.8±3.4), **44** with one piglet with arthritis (*23%*, 12.7±2.9), **38** with more than one piglet with arthritis **(***20%*, 15.3±3.2).
L-boars; **51** litters, **26** with no arthritis (*51%*, 10.6±4.2), **16** with one piglet with arthritis (*31%*, 13.5±2.3), **9** with more than one piglet with arthritis (*18%*, 14.8±2.6).
Y-boars; **284** litters, **175** with no arthritis (*62%*, 11.3±3.5), **63** with one piglet with arthritis (*22%*, 12.7±3.2), **46** with more than one piglet with arthritis (*16%*, 13.9±2.5).
In **H-**, **L-** and **Y**-boars a significantly ($P < 0.01$) larger litter size was seen in litters with more than one arthritis.
Conclusion: There was a higher incidence of arthritis in litters with more than 12 piglets. Although the total milk production of sows tends to increase with litter size the amount of milk per piglet tends to decrease, and it is likely that increased aggressions between piglets increase the risk for skin lesions - especially during the first few days of life.
Still, there were large litters without arthritis. The estimates of genetic correlation to sow and boar found in this study imply that good conformation/locomotion traits could be combined litter size. Leg conformation and locomotion traits are heritable, so it ought to be possible to improve these traits by selection.

Disclosure of Interest: None Declared

Keywords: arthritis, genetic , piglets

Miscellaneous

PO-PW1-009

A case report of tracheitis in finishing pigs in France

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Introduction: During an outbreak of respiratory disease, pigs suffering of severe and usually fatal respiratory distress can be observed sporadically. During necropsy, oedema and haemorrhage of the mucosa can be observed in the trachea leading to the suspicion of severe tracheitis. However, aetiology of tracheitis remains obscure in pigs. We report here a clinical case of tracheitis in finishing pigs.
Materials and Methods: This study reports on a 200 sow farrow-to-finish farm located in Brittany, France. Groups of sows farrow every 2 weeks and piglets are weaned at 21 days of age. The farm is free of PRRSV and *Actinobacillus pleuropneumoniae*. Piglets are vaccinated against *Mycoplasma hyopneumoniae (Mhyo)* at 8 and 28 days of age. In the nursery, piglets are treated with tilmicosine at 300 ppm and flubendazole at 15 ppm in the first feed during fifteen days. When moved in the finishing phase, pigs are orally treated for five days with tylosin, 25mg/kg/day. In January 2015, an outbreak of respiratory disease due to H1N2 swine influenza virus (SIV) occurred. During that outbreak, chronic coughing of pigs without dyspnoea was observed. In addition, some pigs with severe respiratory distress and tracheitis were also found. A clinical investigation including post-mortem examinations and laboratory analysis was initiated.
Results: Clinical signs were observed on heavy fattening pigs including hard/rough coughing, severe dyspnoea and death of pigs left untreated (marbofloxacine, 2mg/kg and/or dexamethasone, 0.03mg/kg). Dark red lesions and consolidation of the cranial lobes of the lungs were indicative of bronchopneumonia and histology confirmed those as enzootic pneumonia lesions due to *Mhyo.* A serological profile (ELISA Oxoid) revealed a seroconversion against *Mhyo* one month before slaughtering. Gross lesions in the trachea showed haemorrhagic lesions, necrotic ulceration, oedema and fibrin, confirmed by histology that revealed as well bacterial infection of the lesions. *Streptococcus suis* type 4 and *Staphylococcus hyicus* were recovered from the trachea. During the investigation, parasitic infestation with *Ascaris suum* was concurrently identified by serology (Serasca®).
Conclusion: Tracheitis clinical signs and lesions were more severe during an outbreak of SIV. In this case, our hypothesis is that tracheitis was triggered by Porcine Respiratory Disease Complex (PRDC), with a cough-associated tracheal damage. Indeed, SIV, *Mhyo* and *Ascaris suum* were identified in finishing pigs and after implementation of measures against those pathogens, clinical signs of tracheitis were lowered.

Disclosure of Interest: None Declared

Keywords: respiratory disease, Tracheitis

Poster Abstracts

Miscellaneous

PO-PW1-010

Improving pathology based disease surveillance in pigs by re-establishing links with the industry

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Introduction: The commercial pig industry in Ireland is the third largest agriculture sector in Ireland, representing 8% of gross agricultural output. It is comprised of 1.5 million pigs based in approximately 329 large integrated farms. The Irish commercial pig industry is highly intensive and while locally concentrated has numerous commercial links abroad. In 2013 the Veterinary Laboratory Service (VLS) of the Department of Agriculture Food and Marine (DAFM) coordinated by Pathology division embarked on an initiative to strengthen links with the pig industry with a view to offsetting risk from endemic and emerging disease threats, arising at farm level and transnationally.
Materials and Methods: VLS have engaged with the main stakeholders in the industry, pig veterinary practitioners, farmers and farming organisations to identify areas where involvement and application of specific expertise could mitigate risk of disease, improve animal health/ welfare and increase farm efficiency.
Results: The provision and development of expertise in anatomical pathology and veterinary laboratory diagnostics for pig diseases was identified as essential to establishing and maintaining durable engagement with pig practitioners and the wider industry, thus forming the cornerstone of effective disease surveillance. Improved communication with private veterinary practitioners allowed gaps in diagnostic capabilities to be identified and steps taken to address these. Applied research projects and targeted surveillance on respiratory disease and neonatal diarrhoea serve to provide important surveillance and biosecurity data on endemic diseases and to demonstrate absence of evidence of diseases which threaten the international pig industry such as porcine epidemic diararhoea.
Conclusion: Since 2013, the strategy adopted by VLS has evolved into productive surveillance collaborations resulting in increased pig carcase submissions to the VLS from at risk animal groupings which is highly significant especially in light of increased disease threats to the pig industry worldwide. Emphasis on applied research, particularly to enhance surveillance and diagnostic capabilities, has forged productive relationships among stakeholders and increased the relevance of the VLS to the pig industry, Such cooperation has enhanced the quality and value of surveillance activities for the industry that will assist the industry to respond to disease threats as they arise.

Disclosure of Interest: None Declared

Keywords: Ireland, Pigs, Surveillance

Miscellaneous

PO-PW1-011

Genome-Wide Association Study of Periweaning Failure-to-Thrive Syndrome (PFTS) in Swine Suggests Genes Involved with Depression

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Introduction: Porcine periweaning-failure-to-thrive syndrome (PFTS) is a worldwide spread condition with unknown etiology that affects newly weaned piglets. The morbidity and mortality rates associated with PFTS range from 1 to 20% in nursery piglets indicating the importance of this emergent swine disease. No direct transmission of this condition has been observed among piglets. In addition, no relationship has been identified with the most common infectious agents occurring in the swine industry and PFTS, and the disease has not been reproduced experimentally yet. Some studies have indicated the importance of genetics on the appearance of PFTS, and proposed the existence of genetic predisposition. In this study, we present the first report of PFTS in South America and the identification or genetic markers associated with PFTS using a Genome-Wide Association Study (GWAS).
Materials and Methods: Piglets used in this study were originated from a terminal cross (AGPIC-337 x DB-25), raised in the State of Santa Catarina, Brazil. Sixty-four piglets (34 healthy and 30 with suggestive clinical signs of PFTS) with 35 days of age were selected to have at least one affected and one healthy animal from the same pen. DNA was extracted from blood samples and genotyped at *Deoxi* Biotecnologia, Brazil, using the Illumina PorcineSNP60V2 BeadChip, which contains 61,565 SNPs across the swine genome. SNPs and samples were tested for their quality prior to the analysis using standard parameters. Tests for population stratification and substructure followed by a GWAS using an allelic test to identify SNPs associated with PFTS were conducted.
Results: After quality control, 49,586 SNPs and 53 piglets remained for the association test (24 cases and 29 controls). Using an allelic test, three regions showed association with the appearance of PFTS: one located on *SSCX* ($p= 5x10^{-5}$), and two other regions on *SSC14* (59,414,987 to 79,124,234 bp) ($p= 2x10^{-4}$). The first region on *SSC14* was composed of one marker located at 59,414,987 bp. The second region on *SSC14* was composed of 12 SNPs in high LD ($r^2 > 0.89$), from 69,709,443 bp to 79,124,234 bp. These regions harbor important functional candidate genes associated with personality behaviors in humans, especially with depression.
Conclusion: The results indicate that PFTS might be involved in neurological disorders affecting susceptible piglets when challenged to a stressful event as weaning. Therefore, our findings could help on the identification of susceptible piglets, becoming an important tool to be used in piglet selection to reduce the prevalence of this illness.

Disclosure of Interest: None Declared

Keywords: Depression, GWAS, PFTS

Miscellaneous

PO-PW1-012

Influence of Supplemental Iron Dextran Injections on Hemoglobin Concentrations and Piglet Growth

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Introduction: To prevent iron deficiency anemia, supplemental iron typically is given to piglets within 5 d after birth. Large piglets are at greater risk of iron deficiency than are smaller piglets; however, iron dextran injection protocols are highly variable. The objectives were to determine the within herd prevalence of anemia among piglets, and to evaluate the influence of a supplemental iron dextran injection on growth.
Materials and Methods: The study (n=436 piglets) was conducted on 5 sow farms and nursery facilities. Two farms injected piglets with 200 mg iron dextran at processing. The third farm used 150 mg iron dextran at the time of processing. Farm 4 used 150 mg iron dextran when pigs were 1 d and 5 d of age. For Farm 5, pigs were injected with iron dextran (150 mg) at processing and on the day prior to weaning. At d 14, 6 piglets/litter were blood sampled and matched by body weight to provide a pair of heavy, medium piglets, and light piglets in each litter. One pig/pair received 200 mg iron dextron (Uniferon®; TMT pigs) immediately after the blood collection. At 3 wk after weaning, all pigs were weighed and blood samples collected. Samples were analyzed for Hb concentrations with a HemacueHB201® instrument. Multiple linear regression models were used for Hb concentration and weight. Since the models suffered from multicollinearity and significant interactions, simple effects of treatment were used for combinations of factors.
Results: Prior to the iron dextran injection at 14 d of age, Hb concentrations were higher (P<.05) in the lighter pigs than in the heavy pigs. At 44 d of age (3 wk after weaning), there were no differences in Hb concentrations between TMT and CON groups. At 44 d of age, differences in BW were similar to those differences at the onset of the study. For Farms 1 and 3, the heavy pigs, treated with iron dextran at 14 d of age, were heavier (P < 0.05) than their control counterparts. The additional iron injection had no effect on growth in Farms 2, 4 and 5.
Conclusion: The heavy pigs had the lowest Hb concentrations at 14 d of age. This inverse relationship, i.e. heavy pigs with low Hb, creates a confounding issue (as seen with interactions) for the evaluation of supplemental iron on pig growth. Pre-planned differences BW continued into the nursery phase of production. The influence on weight gains was not consistent in the 5 farms. It was evident that the BW at 14 d of age had the most significant influence on subsequent BW at 44 d of age. There was considerable variation among pigs (within a weight class) in Hb concentrations. This may reflect shortcomings in injection technique.

Disclosure of Interest: None Declared

Keywords: Hemoglobin, Iron dextran, piglets

Miscellaneous

PO-PW1-013

Gastric emptying is similar between IUGR and normal piglets

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Introduction: Increased litter sizes due to hyper prolific sows have led to litters with up to 30% of piglets being born with signs of intra-uterine growth restriction (IUGR). IUGR piglets have lower survival rates and are more likely to have empty stomachs at 24 hours. In addition, it is estimated that IUGR piglets can only consume 100 g of the recommended 250 g of colostrum within the first 24 hours. The gastric emptying rate of these piglets might influence their survival rates and therefore we hypothesised that the gastric emptying rate would be lower in IUGR piglets compared to normal piglets.
Materials and Methods: Due to the brain sparing effect, IUGR piglets can easily be recognised on-farm by their headshape rather than only by their birth weight. Forty-eight piglets were therefore classified at birth as either normal or IUGR based on their head morphology (24 normal and 24 IUGR piglets). Piglets were removed from the sow before they had suckled, and were marked for identification purposes, dried, and the umbilical cord shortened to 15 cm. Birth weight was recorded and sow's colostrum obtained from other sows previously, warmed to 35°C and tube-fed to all piglets at 12mL/kg body weight as soon as possible after birth (t=0 min). The ambient temperature in the farrowing room was 24°C and piglets were kept in a boarded of creep area (with rape granules) with a heating lamp (150W) and with temperatures ranging from 24-29°C. The piglets were randomly allocated to be euthanized at 15, 30, 60 and 120 min (all groups n=6) after bolus feeding, and the weights of stomach and its content were recorded. Data were analyzed univariately using PROC GLM in SAS.
Results: Average birth weights were 0.7 ± 0.04 kg *vs.* 1.38 ± 0.04 kg in IUGR and normal piglets (P<0.001). After 15 min, approx. 40% of the tube-fed colostrum had passed through the stomach of the normal piglets and approx. 25% of the tube-fed colostrum had passed through the stomach of the IUGR piglets. At 30 min this value was 56% and 45% respectively, at 60 min it was 71% and 67% respectively, and finally, after 120 min these values were 83% and 78% for normal and for IUGR piglets, respectively, of the tube-fed colostrum that had passed through the stomach. The differences between IUGR and normal piglets were not significant.
Conclusion: Gastric emptying rates are similar between IUGR and normal piglets, and at 120 min the ventricles are almost empty after a single bolus feeding.

Disclosure of Interest: None Declared

Keywords: gastric emptying rate, intra-uterine growth restricted piglets, newborn piglets

Poster Abstracts

Miscellaneous

PO-PW1-014

GENOME WIDE-ASSOCIATION STUDY IN ANIMALS AFFECTED BY PERIWEANING FAIL-TO-THRIVE SYNDROME (PFTS). PRELIMINARY RESULTS

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Introduction: Periweaning fail-to-thrive syndrome (PFTS) has been recently described in several countries and is clinically characterized by loss of body weight, anorexia, oral stereotypes and depression. The apparition of the condition is around two first weeks after weaning. The etiology remains unclear but certain individual influence to predisposition has been described; in some cases one single boar produces the majority of sick animals (Ramis et al, 2015). So, the aim of this work was try to find some single nucleotide polymorphism (SNP) related with the PFTS.
Materials and Methods: Forty-eight animals form the same farm were sampled. Among them, 30 were animals affected by PFTS, 10 were contemporaneous normal animals (randomly selected) and 8 were the boars mating in this farm. The PFTS animals were diagnosed on a histopathological basis and major pathogens (PRRS, SIV and PCV2) were discarded by q-PCR and immunohistochemistry.
DNA samples were sent to GeneSeek (USA) in order to run a PorcineSNP60 DNA BeadChip (Illumina®) analysis. This array includes 61,565 SNPs distributed over all the swine chromosomes. The results were included in an R database and analyzed by means of PLINK software. Those SNPs with a frequency lower than 30% of genotyped samples were removed. A Bonferroni adjustment was used.
Results: Finally, after filtered, 51,796 SNPs were used for the association study. None of them showed significant association with PFTS animals, even when in the standard analysis 3 SNPs in chromosome 4 and one in chromosome 9 showed a slight significance. This significant relation disappeared when the Bonferroni's correction was made.
On one hand, it should take into account that this array type very little number of SNPs in chromosome X and practically none in chromosome Y. On the other hand, the sample size is very small and probably increasing it could results in association between some SNP and the disease
Conclusion: With the sample size and the array used it was not possible to establish a relation between any single nucleotide polymorphism analyzed and the PFTS. Probably, more research and increasing of sample size would be necessary to find any SNP related with the syndrome.

Disclosure of Interest: None Declared

Keywords: GWAS, PFTS, SNPS

Miscellaneous

PO-PW1-015

Detection of Tylvalosin in Sows Milk Following Administration of Aivlosin®

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Introduction: Tylvalosin (TVN), a 2nd generation macrolide antibiotic, is the active ingredient in Aivlosin® products administered to swine. Aivlosin® is authorised throughout the European Union and other international markets for the treatment of respiratory and enteric disease in swine. The objective of this study was to investigate whether TVN is present in sow's milk after administration of Aivlosin® in feed.
Materials and Methods: Twenty sows received a small medicated feed ration in advance of their normal non-medicated feed each morning for 5 consecutive days. Daily doses of the medicated feed were administered for 3 days prior to farrowing and for two days after farrowing. The target daily dose was 5 mg tylvalosin/kg body weight.

Milk samples were collected from each sow approximately 2 hours after consumption of the medicated feed ration on the fifth and final day of medication. Milk release was induced by an intramuscular injection of 20 IU of oxytocin. Each milk sample was frozen immediately after collection prior to subsequent TVN analysis using a validated analytical method. The method utilised protein precipitation and solvent extraction prior to LC-MS/MS analysis. The limit of quantification of the method was 100 ng/mL.
Results: Six of the twenty sow milk samples investigated contained TVN concentrations above the limit of quantification (range 141 to 977 ng/mL) and fourteen contained TVN concentrations below the limit of quantification (range 12.0 to 96.1 ng/mL).

Consumption of the small medicated feed ration was not compromised due to the presence of TVN.
Conclusion: This study demonstrated that tylvalosin could be detected in sows' milk after administration of Aivlosin® in feed for 5 consecutive days at a target daily dose of 5 mg tylvalosin/kg body weight. Further studies are required to investigate the disposition of tylvalosin into sows' milk and to evaluate the therapeutic implications of the presence of tylvalosin in milk during the lactation period.

Disclosure of Interest: J. Mora Conflict with: Eco Animal Health Ltd., G. SPEIRS Conflict with: Eco Animal Health Ltd., E. Abbott Conflict with: Eco Animal Health Ltd.

Keywords: Aivlosin, Milk, Sow

Miscellaneous

PO-PW1-016

How to bring science based information to farmers: experiences of the Pig Information Counter

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Introduction: In 2012, the Flemish government founded the "Pig Information Counter" as an answer to pig farmers organizations' request for easily accessible noncommercial information. The mission is to enhance sustainable pig production in Flanders by providing farmers and other members of the value chain with practical and recent information. Three years later, an analysis of the succes factors was made.
Materials and Methods: The core business of the Pig Information Counter is answering farmers' questions in an understandable way. This service is offered free of charge. All answers, together with all relevant research results and best practices are directly made available through www.varkensloket.be, newsletters and publications in pig journals. Symposia and workshops are organized to provide the farmers with relevant information on pig production. The advices are based on (inter)national applied and fundamental research experience and practical knowledge of the Pig Research Network.
Results: Since the spring of 2012, 282 written advices have been communicated to the pork sector. Most questions have been asked by pig farmers (33%), followed by commercial companies (17%), researchers (13%), hobby pig keepers (13%), government (8%), students (7%) and veterinarians (6%). All areas of pig production are handled: farm management (29%), hobby pig husbandry (14%), feed (16%) and drinking water (6%) strategy, manure/environment (9%), animal health (6%), indoor climate (6%), organic pig husbandry (4%), housing (4%), slaughter quality (3%), animal welfare (2%), biosecurity (2%) and reproduction (1%). Time from answer to response is on average 16 days. The website contains now more than 300 publications and counts more than 32,000 visits. For self-evaluation, a questionnaire was sent out to the first 90 questioners to investigate the questioners' perceptions of the advices. The overall response rate was 48%. The great majority of the questioners judged the written feedback and the period of time in which the advice was given positively (88%). Nearly all questioners (98%) indicated that they would recommend the Pig Information Counter to colleagues and thought it was realistic that they would ask another question (93%).
Conclusion: The questions asked to the Pig Information Counter and the answers to the questionnaire confirm that there is a need of objective information ('second opinion'), apart from the information farmers receive through advisors that visit the farm regularly, and that the Pig Information Counter meets these demands. To improve its function, the Pig Information Counter will put more focus on peer to peer learning through farmers' network groups, farm visits and audio-visual material.

Disclosure of Interest: None Declared

Keywords: Farmers, Knowledge transfer, Science based practical information

Miscellaneous

PO-PW1-017

Comparison of Pneumocystis nucleic acid and antibody profiles in two Austrian pig herds

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Introduction: *Pneumocystis* belongs to the opportunistic fungi and is of high clinical relevance in immunocompromised patients who may develop severe interstitial pneumonia. *Pneumocystis carinii* f. sp. *suis* can frequently be detected in pigs co-infected with other respiratory pathogens. The occurrence of *Pneumocystis* on farm level has not yet been investigated. For this reason, the aim of the present study was the evaluation of *Pneumocystis* nucleic acid and antibody profiles in two Austrian pig farms.
Materials and Methods: Bronchoalveolar lavage fluid (BALF) and serum samples of pigs from different age classes (suckling piglets 1st week of life (S1), suckling piglets 3rd week (S2), weaning piglets 2nd month (W1), weaning piglets 3rd month (W2), fattening pigs 4th month (F), and sows (SOW)) of two Austrian farrow-to-finish farms with respiratory disorders were collected. On farm A, 45 BALF samples (S1: 8, S2: 7, W1: 8, W2: 8, F: 14), and on farm B, 47 BALF samples (S1: 8, S2: 8, W1: 8, W2: 8, F: 15) were taken. On each farm, 63 serum samples were collected (S1: 8, S2: 8, W1: 8, W2: 8, F: 15, SOW: 16). BALF samples were analyzed by qPCR, antibody titres against *Pneumocystis* were determined by XpressBio *Pneumocystis carinii* swine ELISA (Express Biotech International, Frederick, MD, USA).
Results: On farm A, 5 of 8 suckling piglets in the 1st week of life were positive and had a mean *Pneumocystis* burden of 10^7 copies/ml BALF. In the groups S2 and W1, all pigs were positive and the concentration increased to 10^9 copies/ml BALF on average. In group W2, only one pig was positive and the *Pneumocystis* concentration decreased to 10^6 copies/ml BALF. In group F, the fungus could not be detected anymore. Farm B showed a different profile. The pigs were negative until the 3rd month of life. In group W2, 4 of 8 pigs and in group F, 8 of 15 pigs were positive, both groups with a mean burden of 10^7 copies/ml BALF. Antibodies against *Pneumocystis* were present in sows, as maternal antibodies in suckling piglets and as immunological reaction after infection.
Conclusion: The results clearly show that the *Pneumocystis* profiles can vary between farms. *Pneumocystis* is an opportunistic fungus and its proliferation is obligatory related to immunosuppression. In the present study, other respiratory pathogens were not analyzed, but we assume that proliferation is triggered by co-infections. Suckling piglets are probably more susceptible to an infection, can be infected very early in life and show higher burdens but, nevertheless, *Pneumocystis* can also contribute to respiratory disorders in older pigs.

Disclosure of Interest: None Declared

Keywords: Pneumocystis carinii f. sp. suis, qPCR, serology

Poster Abstracts

Miscellaneous

PO-PW1-018

Ammonia concentrations in dust samples from a fattening unit with bad air quality

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Introduction: Effects of 5 to 150 ppm gaseous ammonia onto respiratory health and production data in pigs have been described in literature with varying results. Ammonia is known to cause oxidative stress in epithelial cells, to irritate nociceptors and to disturb the mucocillary clearance. In addition, a pH-shift within the epithelial lining fluid covering the airways with the consequence of an efficacy loss of pH-dependent natural antibacterial peptides is assumed. In a fattening unit with low gaseous ammonia concentrations but bad air quality leading to airway irritation, dust samples were analysed for bound ammonia. The hypothesis was, that ammonia bound to inhalable dust particles might irritate the mucosa.
Materials and Methods: Sedimentation dust was sampled on six boards covered with foil in two fattening units with partly slatted floor with 109 and 110 pigs each within two time periods of four and six weeks. Gaseous ammonia was determined by a single gas detector with an accuracy of 1 ppm.
Dust-bound ammonia was quantified photometrically after acidification by the reaction from alpha-ketoglutarate with ammonia to L-glutamate, a method for the quantification of ammonia in plasma with a detection limit of 14.7µmol/l. The variation coefficient in two four-fold determinations in dust samples was 5.4-5.9%.
Results: No respiratory disorders occurred during the examination period and only minor lung lesions were detectable at slaughter checks. Subjective perception of air quality by workers was negative because of airway irritations. Gaseous ammonia was in the range of 0-28 ppm during the examination period. The amount of sedimentation dust was 2.6±0.7 [g/m²/day], which is high in comparison to data from literature. Mean ammonia concentrations were 5.1±1.3 µg/mg dust. With a supposed air dust concentration of 10 mg/m³ this would result in approximately 0.07ppm ammonia in the air.
Conclusion: A relatively high dust load was measured in a fattening unit with partly slatted floor. Not only dust but also dust-bound ammonia might have harmful effects in the respiratory tract. During constant exposure and solubilisation of dust bound ammonia in an approximated volume of 2 ml epithelial lining fluid in the lung, ammonia concentrations with harmful effects shown in cell culture might also occur in the lung. A negative effect of ammonia on phagozytosis of dendritic cells in cell culture has been shown for concentrations of 1.3µg/ml.

Disclosure of Interest: None Declared

Keywords: dust bound ammonia, sedimentation dust

Miscellaneous

PO-PW1-019

Approaches and issues related to measurement of antibiotic use (ABU) data in the US swine industry

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Introduction: The urgency to address antibiotic resistance in human medicine is echoed among health groups at globally. ABU in all arenas (human, food animal, companion animal, aquaculture, agriculture) contributes to the phenomenon of resistance, albeit with variable clinical consequences. Improving antibiotic stewardship is a cornerstone of efforts to combat the emergence of clinical resistance, and measurement of both ABU and resistance is needed. In most countries, current national data based on gross weight of products sold across multiple species lack sufficient granularity to provide meaningful support for stewardship activities.
Materials and Methods: A review of existing systems for measuring ABU was conducted to evaluate potential models for the US industry. This included in person meetings with groups involved with development systems in some European countries, and reviews of published materials in other countries. In parallel, information is being gathered on ABU data kept by US producers, to understand the scope of archived data and the opportunities and barriers to sharing data within the industry and to external stakeholders.
Results: Key issues arising include: 1) Clear definition of the core purpose for measuring ABU (obtaining meaningful and representative data on trends at an industry level vs. monitoring ABU at an individual user level for benchmarking and interventions) and 2) Definition of appropriate metrics for measurement, including the scope of compounds to include (e.g. 'medically important' vs. not). Weight based measures for ABU are essentially meaningless and adjustment for potency is necessary and done in some countries. However, adjusted measures are also problematic and population based measures are not consistently applied across existing programs. There also is a need to differentiate ABU by stage of production. In the highly consolidated US industry, considerable data on ABU is kept privately for cost accounting purposes. Such data are biased towards larger systems, but may represent a large part of commercial production. Efforts are now being directed to design a framework for confidential data sharing and analysis, including the potential of within industry benchmarking and contributing another data stream to national surveillance initiatives.
Conclusion: There is concern that ABU reduction is seen as synonymous with judicious use. Reduction should not be pursued or mandated independent of efforts to assess resistance in human pathogens or without concurrent assessment of the appropriateness of use, and impact on clinical outcomes, and animal health and well-being. Appropriate practices that most benefit animal health need to be defined based on evidence not opinion.

Disclosure of Interest: None Declared

Keywords: antibiotic use, measurement, metrics

Miscellaneous

PO-PW1-020

Evaluation of impulse oscillometry in pigs originating from the field

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Introduction: Impulse oscillometry is a non-invasive method for analyzing lung function and variables of ventilation. Validation of the system for diagnosis of respiratory disease in humans, calves and horses has shown a higher sensitivity compared to classical clinical techniques. One of the main advantages is that pulmonary alterations can be allocated to upper and peripheral airways on the basis of associations with particular frequencies. The aim of the present study was to evaluate impulse oscillometry as an advanced clinical method to analyze lung function in pigs.
Materials and Methods: Fifty-eight German hybrid pigs from 29 different herds were chosen for routine diagnostics in the context of herd health service. Standard clinical examination was complemented by the use of the impulse oscillometry system (IOS), bronchoalveolar lavage and detailed pathology. Bronchoalveolar lavage fluid and lung tissue samples were examined for relevant airway pathogens in swine by molecular and microbiological methods. The reactance area (AX) was evaluated as a new impulse oscillometric parameter, reflecting changes in the degrees of pulmonary restriction and/or peripheral airway obstruction.
Results: Special clinical examination of the respiratory tract included 29 parameters which characterised the pulmonary system thoroughly. Eight clinical parameters, exhibiting a marked relationship with impulse oscillometry, were extracted via multiple regression analysis. The linear relationship between clinical findings and variables of respiratory mechanics had a mean r^2 of 0.52. Three IOS categories were created based on current IOS data. The severity of pulmonary dysfunction increased from category 1 to 3 which was in concordance with clinical findings. In general, clinical findings deteriorated with increasing impairment of lung function. Clinical parameters characterising the lower respiratory tract closely correlated with established IOS indices predominantly representing peripheral airways. The correlations between clinical findings and AX were similar to established IOS parameters, indicating the ability of AX to qualitatively and quantitatively assess functional disorders in the lung periphery.
Conclusion: High repeatability and a significant interindividual variability of test results suggested IOS as a potential method for complementing clinical diagnostics in pigs regarding respiratory diseases. However, this variability is only partially explained by clinical findings. This provides evidence for a deeper impact of IOS with regard to pulmonary tract diseases. Histopathological data will be needed to provide a complete understanding of IOS data and variability.

Disclosure of Interest: None Declared

Keywords: Diagnostics, PRDC

Miscellaneous

PO-PW1-021

Survey of pulmonary lesions and pleuritis in slaughter-aged pigs in Italy

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Introduction: Cranio-ventral pulmonary consolidation (EP-like lesions) and chronic pleuritis (CP) are common findings in slaughtered pigs. Pleural lesions involving dorso-caudal lobes are suggestive of pleuropneumonia due to *Actinobacillus pleuropneumoniae* (App). The aims of the present study were to investigate the prevalence of lung and pleural lesions at abattoirs in Italy and to identify major factors potentially associated with the prevalence and the severity of the lesions.
Materials and Methods: The lungs of 4896 pigs, 9 to 10 months old, belonging to 50 Italian herds (25 vaccinated and 25 unvaccinated for App) were scored at the slaughterhouse for lung and pleural lesions according to Madec's grid and to the Slaughterhouse Pleuritis Evaluation System (SPES) respectively. For each batch the EP-like average value, the SPES average value, i.e. the sum of each lung score/number of scored lungs, and the APP index, i.e. the frequency of pleuritis lesions with a SPES score ≥2 in a batch*mean pleuritis lesion score of animals with SPES ≥2 were calculated. Twenty blood samples were collected at 10, 18 weeks of age and at slaughter from each batch and tested for *M. hyo* (ELISA IDvet), App-ApxIV, PRRS (ELISA IDEXX) and SIV (HI test for H1N1, H3N2, H1N2). Each farm was visited by a swine veterinarian and data about farm characteristics, herd size, pig flow, type of floor and ventilation, description of RD on the farm, history of pleuropneumoniae in the previous 2 years and vaccination programs were collected using a questionnaire. The association between the questionnaire results, seroprevalence and lung lesions were analyzed using non-parametric tests.
Results: EP-like lesions were detected in 2188 (44.7%) lungs and the average value was 0.95 (st. dev. 0.38). Chronic pleuritis were recorded in 2614 (53.4%) lungs. Dorso-caudal pleuritis, suggestive of recovered pleuropneumonia, was found in 1214 lungs (24.8%). The mean SPES value of all lungs was 0.8 (st. dev. 0.35). The mean APPI of all studied batches was 0.6 (st. dev. 0.35). The mean SPES and APPI values per batches were statistically associated with App seroprevalence ($P<0.05$, Pearson r 0.28 and 0.37 respectively) and higher in farms with history of App ($P<0.05$) isolation.
Conclusion: Chronic pleuritis and cranio-ventral pulmonary consolidation are frequently observed in pigs at abattoirs in Italy, suggesting a detrimental economic effect on pig production. The results of this study highlight that a history of App isolation and its seroprevalence are risk factors for dorsocaudal chronic pleuritis.

Disclosure of Interest: None Declared

Keywords: None

Poster Abstracts

Miscellaneous

PO-PW1-022

QUANTIFICATION OF PORCINE ENDOGENOUS RETROVIRUSES COPIES IN THE DNA OF ANIMALS SUFFERING PORCINE FAIL-TO-THRIVE SYNDROME (PFTS)

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Introduction: Periweaning fail-to-thrive syndrome (PFTS) has been recently described in several countries and its etiology remains unclear but certain individual influence to predisposition has been described (Ramis et al, 2015). On the other hand, even without any reference in literature, has been suggested that could be some relation with Porcine Endogenous Retroviruses (PERVs). The PERVs are ancient DNA inserted in the pig DNA and potentially can results in the transcription of mRNA and the formation of a virus. The aim of this work was try to find some relation with the number of PERVs inserted in the porcine DNA of PFTS and normal control animals.
Materials and Methods: PFTS animals were identified and diagnosed in two farms in Central (CS) and North Spain (NS). The diagnosis was done on the basis of histopathological findings and major pathogens (PRRS, SIV and PCV2) absence, demonstrated by q-PCR and immunohistochemistry. The animals were sampled to obtain blood from sick animals (n= 10 in CS and n=30 in NS) and normal randomly selected animals (n=6, n=10, respectively). Moreover, the boars mating in both farms were sampled (n=5 and n=8, respectively)
DNA was isolated from the blood samples and a q-PCR was performed. The primers used were those designed by Ma et al. (2010), which are specific for the *pol* gene, present in PERV-a, PERV-B and PERV-C subtypes. The quantification was normalized to the initial quantity of DNA isolated and assuming that every cell has 6 pg of DNA was calculated the number of copies by cell. Data were statistically analyzed using a Mann-Whitney's U test.
Results: The average number of PERVs inserted was 69±46 and 4,532±1,884copies/cell for PFTS and normal animals in CS (p=0.028), and 3,903±1,191 and 5,208±1,502 in NS, but in this farm the difference was not significant. Interestingly, in both cases the number of copies was lower in PFTS sick animals than in normal randomly selected animals.
Conclusion: The PFTS animals showed a lower number of PERVs inserted in their DNA. This, obviously, doesn't mean that a low number of PERVs can be related with the disease but, could be an indicator that some individuals have higher predisposition to suffer the disease.

Disclosure of Interest: None Declared

Keywords: PFTS, PORCINE ENDOGENOUS RETROVIRUS

Miscellaneous

PO-PW1-023

Increased weaning weight and higher performance with acid booster

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Introduction: Growing litter sizes on commercial swine farms are a fact, which leads to new challenges. Within growing litters the diversification is bigger and the animals are in average smaller at weaning. Aim is to achieve a minimum weaning weight of at least 5.0 kg after a three-weeks suckling period and average daily gain of around 220 g during the lactation period. In addition also the milk yield is influenced by the litter size – the bigger the litter size, the higher is the average milk yield per litter, but the smaller is the amount of milk per piglet. On this purpose a product including a blend of formic, acetic and propionic acid, a phytochemical and a permeabilizing complex (NGP, Biotronic® Top3, BIOMIN, Austria), was used in an experiment to test the effect on piglets' growth and their sows' performance.
Materials and Methods: In the experiment 24 sows and their suckling piglets were assigned to two groups with 12 sows each and 146 piglets in control group and 133 piglets in trial group (NGP). The beginning of the trial was at day 80 of gestation and ended after a three-weeks suckling period; in total the trial period was 56 days. The sows were fed commercial gestating and lactating sow diets. The diet of the control group contained no antibiotic or natural growth promoter products, whereas the diet of NGP was supplemented with 2.0 kg NGP/t of feed.
Results: The weaning weight of the piglets after a three-weeks suckling period was significantly ($p<0.01$) higher for the NGP group (5.94^a kg ± 1.5) compared to the control group (5.46^b kg ± 1.4). Also the average daily gain of the piglets was significantly ($p<0.01$) higher in the NGP group (258^a g ± 55) compared to the control group (223^b g ± 63). Looking at the sows' performance, the milk yield of the sows was higher in the NGP group (10.6 kg/d/sow ± 1.9) compared to the control group (10.2 kg/d/sow ± 1.2).
Conclusion: Supplementing the diet of sows with NGP enhanced piglet performance, showing significantly increased average daily gain ($p<0.01$) of the piglets in the NGP group compared to the control group during the suckling period and also significantly increased weight ($p<0.01$) of the piglets in the NGP group compared to the control group at weaning. Milk production of the sows was also positively influenced by the NGP.

Disclosure of Interest: None Declared

Keywords: acid booster, piglet performance

Miscellaneous

PO-PW1-024

Multiplex testing for PRRSV, SIV, and PCV2 antibodies using a Multiplexed Fluorometric Immuno Assay (MFIA)

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Introduction: Porcine Respiratory Disease Complex is a significant problem for the swine industry. It is caused by the interaction of multiple non-infectious and infectious factors, including PRRSV, SIV, and PCV2. Measurement of antibodies to these agents is routinely performed for monitoring herd status or optimizing vaccination protocols. Testing for all these agents at the same time in a single assay could potentially save a lot of labor, time, and cost compared to traditional serological methods.
Materials and Methods: Antigens (ag): recombinant proteins from PRRSV type 1 and 2, AIV, and PCV2 were expressed in E. coli (PRRSV, PCV2) or using Baculovirus (AIV). The purity of the recombinant proteins was evaluated using SDS-PAGE. The identity of each protein was further confirmed by Western blot analysis with anti-His, PRRSV, AIV or PCV2 antibodies.
Ag coupling: ag were coupled to 4 different sets of magnetic beads (Magplex®, Luminex) using proprietary methods. For each ag, the optimal conditions providing the highest signal-to-noise ratio were determined.
1-plex MFIA: all incubations were done at room temperature in the dark on a shaker. Diluted samples were incubated for 1 hour with relevant antigen-coated bead suspension. After serial washings biotinylated goat anti-swine IgG was added and the plates incubated for 30 minutes. The plates were further washed prior to adding streptavidin – phycoerythrin and incubated for 30 minutes. After final washings the beads were suspended in assay buffer and the plates were read using a dual-laser instrument (LX200®, Luminex). Assay parameters were optimized to provide the most consistent and reproducible results.
4-plex MFIA: once the four 1-plex assays worked properly the 4 bead suspensions were mixed and used in a 4-plex assay. Possible interaction between ag and non-immune binding were examined using selected non-immune, homologous and heterologous immune serum controls.
ELISAs: samples were also tested using the PRRS X3 Ab, the Influenza A Ab (IDEXX) and the Ingezym Circo IgG (Ingenasa), .
Test evaluation: test sensitivity and specificity were evaluated using serum samples of known status.
Results: Results obtained with the 4-plex MFIA were very similar to those obtained with the corresponding ELISAs for PRRSV type 1 and 2 and SIV. By contrast a poor correlation was noticed for PCV2.
Conclusion: This MFIA appears to be an interesting alternative to traditional ELISAs for PRRSV and SIV as it offers similar diagnostic performances while saving labor, time, and cost.

Disclosure of Interest: A. Broes Conflict with: Biovet, I. Caya Conflict with: Biovet, M. Bertrand Conflict with: Biovet

Keywords: MFIA, multiplex, serology

Miscellaneous

PO-PW1-025

Rectal temperatures in IUGR piglets under four different conditions

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Introduction: Selection for improved prolificacy in sows has increased litter sizes at birth but has also resulted in an increased percentage of piglets that have been exposed to varying degrees of intra uterine growth restriction (IUGR). These IUGR piglets can be easily recognised on their headshape due to an asymmetrical development of the fetal organs (brain sparing). IUGR piglets are more likely to maintain a low rectal temperature after birth after the initial temperature fall and this might influence their chances of survival. We hypothesised that supplement of colostrum and/or heat from an external source would have a positive effect on the rectal temperature 8 hours post partum.
Materials and Methods: Eighty-four IUGR piglets were randomly allocated one of four treatments in a 2x2 factorial design (with/without supplement and with/isolated from sow). The four treatments were therefore; piglets that stayed with the sow, 1) without colostrum supplement, 2) with colostrum supplement and piglets that were isolated from the sow with an external heat source 3) without a colostrum supplement, 4) with a colostrum supplement. Piglets were classified at birth as IUGR piglets based on their head morphology and randomly allocated a treatment (n=21). Piglets were removed from the sow before they had suckled, numbered and dried. Initial rectal temperature was recorded and piglets were tube-fed warmed porcine colostrum to 35°C at a dose of 12 mL/kg body weight at birth (time=0). Piglets in the 2 treatments without sow were placed under a heating lamp (150W) with a temperature range of 35 – 39°C. Rectal temperature was measured again at 1, 2, 4, 6 and 8 hours after birth. The temperature in the farrowing room was 23°C. Data were analyzed using PROC GLM in SAS.
Results: There was an interaction between time and colostrum supplementation (P<0.001) and between time and piglets with/or isolated from sow (P<0.001). One hour after birth the piglets supplemented with colostrum had a higher rectal temperature (37.5°C) compared to piglets without supplementation (36.6°C; P<0.001). One hour after birth the piglets that had been isolated from the sow had a higher rectal temperature (37.8°C) compared to the groups with sows (36.3°C; P<0.001). Four hours after birth rectal temperature was not affected by treatments.
Conclusion: Porcine colostrum supplementation increased rectal temp one whole degree Celcius an hour after birth. Piglets that were isolated from the sow and kept under external heat also had a higher rectal temperature of a whole degree Celcius compared to piglets that were with the sow. However, after four hours no differences were found between the treatments and our hypothesis is therefore rejected.

Disclosure of Interest: None Declared

Keywords: colostrum supplementation, intra-uterine growth restricted piglets, rectal temperatures

Poster Abstracts

Miscellaneous

PO-PW1-026

Urolithiasis in swine: Case report

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Introduction: The urolithiasis is characterized by the formation and presence of crystallized sediment in the urinary tract. The obstruction begins with inquietude, twitching and urinary incontinence. Advanced stages of the disease are characterized by anorexia, abdominal discomfort and isolation. The urethra and urethral process present inflammation with pus and necrosis by the presence of the calculi (urinary stones). The course of the disorder can last one to two weeks, and in more severe cases it can evolve to death. This summary describes the clinical signs and the results of tests to confirm this affection.
Materials and Methods: A male of swine, Duroc, with about seven years old, raised in picket, fed with concentrate feed and pasture. After three months of castration, the animal was taken to the veterinary hospital with mobility difficulties complaints two weeks ago, anorexia, urine with blood, urine accumulation in the prepuce and necrotic prepuce tissue. Physical and chemical examination of the urine, necropsy and qualitative examination of uroliths were performed.
Results: In urinalysis, the results obtained were: red color; fetid odor; turbidity; density 1,030; volume 4; sediment: erythrocytes + + + + +; squamous cells; for amorphous crystals + + + +; bacteria ++. On 09.10.2015 the animal died and the post mortem evaluation showed a partial necrosis of preputial diverticulum, canal partially obstructed by small multilobed calculi on the extent of the urethra, full penile necrosis, urinary bladder full of bloody urine and calculations (+30), pulmonary edema and severe stomach ulcers. The definitive diagnosis was cystitis with total obstruction of the urethra.
In physical analysis of uroliths the results were: countless, rounded shape, irregular / porous surface, located in the urinary bladder and urethra, Straw color, size ranging from 0.05 mm to 6,99mm. In the chemical analysis were observed carbonate (+); oxalate (-), calcium (+), magnesium (-), urate (-), cystine (-), ammonium (+), phosphate (+) characterizing mixed uroliths calcium carbonate and ammonium phosphate.
Changes in urine pH affect the solubility of some solutes, being the alkalinity conducive to the formation of mixed carbonate and phosphates calculi. Calcium carbonates are reported in animals raised on rich grasslands oxalate. Finishing pigs usually receive rich concentrate feed and feature urinary stones most commonly composed of magnesium phosphate, ammonium and calcium.
Conclusion: According to the case report, it is possible to conclude that the urolithiasis was caused by a supersaturated urine, related to an unbalanced diet and low water intake.

Disclosure of Interest: None Declared

Keywords: urinalysis, urinary tract, uroliths

Miscellaneous

PO-PW1-027

Effectiveness of iron-dextrans and gleptoferron (Gleptosilr) on iron serum biochemistry in piglets

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Introduction: Piglets are born with limited iron reserve and they need supplementation by iron in order to synthetize haemoglobin required for the prevention of anaemia and for proper immune functions. In majority of studies mainly haemoglobin and haematological profile is measured, parameters describing body iron status are missing. The aim of the study is to compare the effectiveness of three iron-containing products (two iron-dextrans and a gleptoferron) on serum biochemistry linked to iron metabolism.
Materials and Methods: Four sows and their litters were used. Piglets were randomly divided into four treatment groups and ear-marked for individual identification. Each treatment was then represented in each litter. On day 3, piglets were given intramuscular injection of two different iron-dextran products (group A, B) and group C was treated by gleptoferron product (Gleptosil) according manufactures recommendation. Control group was not treated with any iron. Groups were consisting of 12 piglets. Blood was collected on day 14 and 28 (weaning age). Iron and total iron-binding capacity (TIBC) were determined by colorimetric method on an AU5822 chemistry analyser (Beckman, Olympus). Percentage of Transferrin saturation (TSAT) with iron was calculated according to the following formula: TSAT= (iron/TIBC) x 100.
Results: On day 14 and 28 all groups had significantly higher serum iron and TSAT and significantly lower TIBC in comparison with control group. Despite the fact that there were no significant differences among iron-treated groups on weaning (day 28), best results were obtained in group C (Gleptosil). Gleptosil group showed highest Fe concentration (µmol/l) 18.55 (±9.34) in comparison with 17.81 (±14.95); 13.13 (±8.99) in group A and B, respectively. Transferrin saturation (%) was again highest in Gleptosil group (36.18±23.29) in comparison with other groups A, B (26.58±29. 50) and (18.78±15.66). TIBC (µmol/l) parameter was best in Gleptosil group 76.22 ±54.04 (group A and B were 89.43±32.13 and 89.44 ±41.62, respectively).
Conclusion: Serum biochemistry and parameters related to iron metabolism are not very frequently evaluated in piglets.. Fe concentration, TIBC parameter and TSAT parameter could be very useful indicators of potential anaemia and quality of iron supplementation in piglets. In our study all treated groups showed significant difference in mentioned parameters in comparisons with control group and piglets injected by gleptoferron (Gleptosil) showed best parameters characterized body iron status.
This work was supported by project LO1218 under the NPU I program (Ministry of Education, Youth and Sports of the Czech Republic).

Disclosure of Interest: None Declared

Keywords: Anemia, biochemistry, Gleptoferron

Miscellaneous

PO-PW1-028

Degrading potential of insemination catheters by composting

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Introduction: Swine industry use massive quantities of insemination catheters to accomplish the reproduction goals required for the services area. However, once these residues are eliminated without the inactivation of the pathogens fixed on it, they must be considered as infective residues with capacity to damage the individual or herd health and in addition, to pollute the environment. Composting is a semi-natural process with capacity to effective degrade organic residues generated by agricultural activities, however the effect of it, on degradation of some natural or synthetic polymers, is not probet yet.
Materials and Methods: Along the symmetry axis of a 60 kg biopile made with mixed biosolids and sawdust, as raw material, were introduced sow insemination catheters manufactured in corn polymer (CP) or made of synthetic polymer (SP). The moisture level of the biopile was maintained in 60 %, and the heap was hand turned each 2 weeks. Along the longitudinal axis of the pile, the two type polymer catheters were co-composted. In days 0, 14, 28, 42 and 56 after of the mixage of the raw material, catheters were retrieved and sections of them were prepared (coated with gold ions) to further been observed using a scanning electronic microscope (Jeol 2511; Japan) using different magnifications. From both types of catheters only were showed the microphotographs, taken in day 56 of composting.
Results: Were observed imperfections on the surface of the SP catheters, but these were consider as a result of the manufacturing process. However, these can allow, the fixation of pathogen microorganisms. In contrast, on the surface of some sections of the CP catheters, was formed a thin layer of biofilm, as a result of the concerted microbial activity (Quorum sensing) allowing the substrate decomposition, that is increased by the formation of additional microfractures, due to exposition of fresh borders of polymer, where the microbial community can form more biofilm.
Conclusion: It is necessary to realize further research to show the different decay rate of the catheters used in swine farms. Composting must be considered as an efficient process to degrade the corn polymer catheters. This result allow in the future, the inactivation or at least the biocontention of the pathogen agents located in such residues, avoiding the dissemination of diseases, and the damage of the herd health in any other operation.

Disclosure of Interest: None Declared

Keywords: Composting, degrading, insemination catheters

Miscellaneous

PO-PW1-029

Reverse heating system for chilling pigs in summertime

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Introduction: Climatic inadequacies may affect welfare and performance of pigs. The aim of this study was to evaluate the potential of running the heating system backwards during the summer with the aim to accomplish a more comfortable indoor climate.
Materials and Methods: The study was made in herd with a module stable for fatteners with a natural mechanic ventilation system. Each unit had two pens sized 19.6 m^2, out of which 5.7 m^2 was a dunging area located outdoors. Each pen housed 20 pigs (0.98 m^2 per pig). Pigs entered the stable at 12 weeks of age and were reared to market weight all in-all out.
Summer batches from two years were compared. A heat exchanger with a high effect (Alfa Laval 70 kW; Δt = 10 °C) was installed and connected to the water-based heating system of floors, running from the 28th of May in 2013 and from the 22nd of April in 2014. The water-flow capacity was 3 m^3/day in 2013 and 15 m^3/day in 2014. Temperature and relative air humidity were monitored and Temperature-Humidity Indexes (THI) were established.
Four pens were videotaped every Tuesday during the entire fattening period. The photos at 08.00 and at 16.00 were used to count the number of pigs at the lying area. They were also used to score the level of contamination of the lying area (0=clean, 1= dung / urine in >25% of the pen, 2= <50%, 3 = >50%).
Results: The summer 2013 was colder, but the mean temperature outdoors (20.2±3.1 vs. 21.6±6.0) and indoors (2013, 23.6±1.7 vs. 24.8±2.5) ranged within 1.5°C. Still, the mean floor temperature was 1°C lower in 2014 (25.2±1.0 vs. 26.3±0.8). The lying area was cleaner in 2014 than in 2013 ($P<0.001$; both am and pm).
The mean indoor temperatures were constantly 1°C higher in the late afternoon. Thus, also the highest THI-values were recorded in the afternoons. Still, the lying area were cleaner in the afternoon than in the morning during both years (2013, 0.57±0.7 vs. 1.23±1.0, $P<0.001$; 2014; 0.13±0.3 vs. 0.32±0.6, $P<0.05$). In 2014, more pigs were at the lying area, both at morning (3.0±1.6 vs. 2.3±2.4, $P=0.01$) and afternoon (4.4±2.3 vs. 3.7±2.8, $P<0.001$).
Conclusion: Growing pigs prefer chill during warm summer days. An effective chilling floor effectively improved the pen hygiene as more pigs used the lying area instead of the slatted dunging area for resting. They thereby avoided the dunging area with the aim to search chill from manure and urine when resting.

Disclosure of Interest: None Declared

Keywords: chilling, hygiene, pigs

Poster Abstracts

Miscellaneous

PO-PW1-030

Multiplex testing for APP 1-9-11, 2, 3-6-8-15, 4-7, 5, 10, and 12 antibodies using a Multiplexed Fluorometric Immuno Assay (MFIA)

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Introduction: *Actinobacillus pleuropneumoniae* (APP) remains an important swine respiratory pathogen. Fifteen APP serotypes corresponding to nine serogroups have been identified so far (1-9-11, 2, 3-6-8-15, 4-7, 5, 10, 12, 13, and 14). The surveillance of swine herds for APP mostly relies on the detection of serotype/serogroup specific antibodies in serum samples. Various serological assays have been developed for that purpose. The most sensitive and specific are indirect ELISA using highly purified long chain lipopolysaccharides (LC-LPS) as antigen.Testing for several serotypes/serogroups at the same time in a single assay could save a lot of labor, time, and cost compared to traditional serological methods.
Materials and Methods: Antigen coupling: highly purified LC-LPS APP1-9-11, APP2, APP3-6-8-15, APP4-7, APP5, APP10, and APP12 antigens were coupled to seven different sets of magnetic beads (Magplex®, Luminex) using a proprietary method. For each antigen, the optimal antigen concentration and coupling conditions (bead concentration, buffers, pH, incubation time, etc.) providing the highest signal-to-noise ratio were determined
1-plex MFIA: all incubations were done at room temperature in the dark on a shaker. Diluted samples were incubated for 1 hour with relevant antigen-coated bead suspension. After serial washings biotinylated goat anti-swine IgG was added and the plates incubated for 30 minutes. The plates were further washed prior to adding streptavidin – phycoerythrin and incubated for 30 minutes. After final washings the beads were suspended in assay buffer and the plates were read using a dual-laser instrument (LX200®, Luminex). Assay parameters were optimized to provide the most consistent and reproducible results.
7-plex MFIA: once the four 1-plex assays worked properly the 7 bead suspensions were mixed and used in a 7-plex assay. Possible interaction between ag and non-immune binding were examined using selected non-immune, homologous and heterologous immune serum controls.
APP ELISA: samples were also tested using the Swinecheck APP1-9-11, APP2, APP3-6-8-15, APP4-7, APP5, APP10, and APP12 ELISA kits (Biovet, Canada).
Test evaluation: test sensitivity and specificity were evaluated using serum samples of known status.
Results: The 7-plex MFIA for APP1-9-11, APP2, APP3-6-8-15, APP4-7, APP5, APP10, and APP12 demonstrates diagnostic performances very similar to those of the corresponding LC-LPS ELISA.
Conclusion: This MFIA appears to be an interesting alternative to traditional ELISAs for APP1-9-11, APP2, APP3-6-8-15, APP4-7, APP5, APP10, and APP12 as it offers similar diagnostic performances while saving labor, time, and cost.

Disclosure of Interest: A. Broes Conflict with: Biovet, I. Caya Conflict with: Biovet, M. Bertrand Conflict with: Biovet

Keywords: MFIA, multiplex, serology

Miscellaneous

PO-PW1-031

Radiography, computed tomography and magnetic resonance imaging of pig head

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Introduction: The pig has become a very popular animal model for wide spectrum of biomedical research because of its morphological and physiological similarity to a human. Therefore, it can be useful for several medical, pharmaceutical and other sectors where the experiments on animals are necessary. The main aim of our study is to evaluate the anatomical structures in the head region of the pig and prepare the manual for next practical clinical usage or pre-clinical research in the future. We focused on three different commonly used diagnostic imaging methods: radiography, computed tomography (CT) and magnetic resonance imaging (MRI).
Materials and Methods: The heads of 24-month-old sows were used. All procedures were conducted following a protocol approved for the collection of samples in the Czech Republic. The Radiography and CT examination of pig heads were performed at the Department of Diagnostic Imaging, UVPS (Brno, CZ). The CT scans were taken using a 16 multislice CT scanner (LightSpeed, GE Healthcare, Milwaukee, Wisconsin, USA). The MRI analysis was performed in the Institute for Clinical and Experimental Medicine (Praha, CZ). Forty-eight transversal and sagittal T1-weighted images were used.
Results: A serial images of individual craniofacial regions were achieved using radiography, CT and MRI imaging methods. Lateral radiographic projections of the porcine head discerned well all bony structures in the facial area. All structures that were identified on radiographs could be identified also on CT scans. On MRI images, we could recognize detailed structures of the nasal cavity and paranasal sinuses similar to the CT scans. Bones and bony processes could be identified by MRI, but details were not easily discernible. Soft tissue structures such as the mucosal covering of the conchae as well as complete structures of the ectoturbinalia and endoturbinalia of the labyrinthus ethmoidalis are shown in clear details.
Conclusion: The comparison of used imaging techniques exhibited differences in the value of providing information. Radiographic imaging is suitable only for general evaluation of the facial area of the pig skull because of thick layer of adipose tissue, which makes the imaging of caudal parts questionable. CT images showed excellent spatial definition of bony structures of the craniofacial area and this technique is very useful for revealing morphological details of mineralized tissues. MRI is especially suitable for soft tissue analysis and the detection of subtle pathologic changes in both bony and soft tissues. Therefore, it is always necessary to consider suitability of individual method according to their future usage.

Disclosure of Interest: None Declared

Keywords: computed tomography , magnetic resonance , radiography

Parasitic Diseases

PO-PF3-005

Risk factors associated with excretion of Ascaris suum eggs in loose housed sows and gilts

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Introduction: Loose housing systems for sows may have increased the occurrence and importance of Ascaris suum. Anthelmintic treatments can result in resistance development. Therefore identification of risk factors and development of alternative control strategies is relevant.
The objective of this study was to identify risk factors associated with Ascaris suum egg counts in sows and gilts from intensive farms with loose housed sows.
Materials and Methods: Twenty sow farms (450 – 2500 sows) were selected from different regions of Denmark. Eight of the farms used routine anthelmintic treatment of breeding animals and 12 farms only performed anthelmintic treatment when considered necessary based on the results of faecal egg counts. From 2012 to 2015, faecal samples were collected from the individual farms at three to five time points. The faecal samples were obtained from 10 gilts and 10 sows at each time point. Nematode egg counts were determined using a McMaster technique. Poisson-regression was used to investigate risk factors associated with A. suum egg counts. Farm was included as a random effect, and fixed effects with a p-value below 0.05 was included in the final model. All statistical analyses were performed using Stata IC 13.
Results: Egg counts were obtained from 784 gilts and 767 sows were included in the statistical analysis. A. suum eggs were detected in 20% of the faecal samples and egg counts were significantly different between breeding animals sampled at different time-points within the same herd ($p<0.001$). Egg counts were significantly higher in breeding animals excreting Oesophagostomum spp. eggs ($p<0.001$) and in sows compared to gilts ($p<0.001$). Routine anthelmintic treatment of breeding animals ($p<0.55$) and herd size ($p<0.48$) were not significantly associated to egg counts.
Conclusion: Breeding animals in large herds did not have higher egg counts, indicating that increasing herd sizes are not causing increasing problems with A. suum despite the use of loose housing systems for gestating sows.
Breeding animals having an Osophagostomum spp infestation were excreting a higher number of eggs suggesting that similar risk factors are associated with the occurrence of both parasites.
Egg counts for A. suum were not different between animals in herds with or without a routine use of anthelmintic treatment of breeding animals. This indicates that monitoring egg counts in faecal samples combined with anthelmintic treatments when necessary can be used to control infestation with A. suum.
The higher egg counts in sows compared to gilts indicate that sows are important targets for such monitoring procedures and anthelmintic treatment strategies.

Disclosure of Interest: None Declared

Keywords: None

Parasitic Diseases

PO-PF3-009

Case report of "ARPEGE" mange eradication program implemented on 5 French farrow-to-finish pig farms

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Introduction: Mange (*Sarcoptes Scabiei* var. *suis)* remains an important disease impacting pig herd productivity. Its prevalence has clearly declined due to successful herd eradication programs. This abstract reports the results obtained on 5 French farms following the implementation of the "ARPEGE" (Approche Raisonnée chez le Porc de l'Eradication de la Gale et de ses effets Economiques) mange eradication program combining IVOMEC® 1% injectable (Merial, France) with IVOMEC® Premix 0.04%.
Materials and Methods: The test sites were 200- to 500-sow well-managed farms operating in farrowing batches. Mange was evidenced both by pruritus and dermatitis at slaughter scores and Mange ELISA evaluation. The "ARPEGE" program was set-up by 8 French vet practitioners. It included biosafety measures, a combined regime of injectable and in-feed ivermectin as follows: sows, gilts, boars and suckling piglets were injected with IVOMEC 1% injectable on D0 and D14. Feed of weaners and fatteners and pigs was supplemented with IVOMEC Premix 0.04% twice for 7 days from D0 and from D14. Pigs housed in hospital pens were also treated and piglets born after D0 were treated once. From D0, a systematic routine mange control was also made at entrance: IVOMEC 1% injectable twice 14 days apart.
Scratching index in sows and dermatitis scores in fatteners at abattoir were measured just before the start of the program and from 6 months to 2 years after. A scratching index (SI) was calculated as the average number of scratching episodes per sow for 15 minutes (SI<0.1: mange absent or under control). At abattoir, a dermatitis score (ADS) was calculated for erythematous papular dermatitis (0 to 3) according to previously described method, reflecting the severity of the skin lesions (ADS<0.5: freedom from mange or mange under control). At the end of the program monitoring, a specific ELISA (UGhent) was also conducted in 40 animals per farm for final confirmation of mange eradication.
Results: The average scratching index in sows decreased from 0.38 [0.24 to 0.64] to 0.06 [0.03 to 0.09], $p<0.01$. The average dermatitis index in fatteners also clearly decreased from 0.55 [0.27 to 0.85] to 0.07 [0.03 to 0.12], $p<0.01$. Anti-*Sarcoptes* antibody titers became all negative, indicating the absence of further contact with the antigen, thus absence of subclinical mange.
Conclusion: Under the conditions of the study, the implementation of "ARPEGE" program combining biosafety measures and IVOMEC 1% injectable with IVOMEC Premix 0.04% was shown to be effective for a rapid and sustained eradication of mange.
The authors thank Dr Alno, Gestin, Glattleider, Langlois d'Estaintot, Le Coz, Maréchal, Simon & Serrano for their contribution to the setting-up of the program.

Disclosure of Interest: B. Boivent Conflict with: Merial S.A.S., G. Perreul Conflict with: Merial S.A.S., O. Merdy Conflict with: Merial S.A.S., R. Arena Conflict with: Merial S.A.S., F. Joisel Conflict with: Merial S.A.S.

Keywords: eradication, ivermectin, mange

Poster Abstracts

Parasitic Diseases

PO-PF3-132

Efficacy of Pigfen® against adult stages and egg excretion of Ascaris suum.

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Introduction: *A. suum* (large round worm) is the most economically important helminth in pigs. An infection leads to lower productivity as it pulls down technical parameters, causes rejected livers due to white liver spots and enhances other diseases. The aim of this study was to evaluate the efficacy of Pigfen® - a new 4 % formulation of fenbendazole - in an induced infection model. The reduction in number of adult worms and in faecal egg count were the two criteria to be considered.
Materials and Methods: Two groups of 20 pigs, weighing 14.6 to 24.3 kg, received approximately 333 embryonated *A. suum* eggs during 3 consecutive days (day -1, 0 and 1). Prior to this study, all pigs were helminth naïve and never received any anthelminthic before. Both study groups were balanced by randomization to ensure homogeneity with regard to sex and bodyweight. 45 Days after infection all pigs from the first group were individually treated with a total dose of 5 mg fenbendazole per kg bodyweight, mixed in 20 % of the daily feed ration. The second group was an untreated control group. On days 45, 47, 49 and 51 after infection, faecal samples of each individual pig were collected rectally to determine egg counts per gram of faeces (EPG). 52 Days after infection, animals were necropsied to count the number of adult worms in the small intestine.
Results: In the untreated group adult *A. suum* worms were recovered from 17 of the 20 pigs. The mean worm count in this control group was 49.85 indicating an adequate infection of the study animals. In the treatment group the anthelminthic efficacy (reduction in number of adult worms) was calculated to be 99.9 %. At the time of the treatment, 7 pigs from the control group and 11 pigs from the treatment group were excreting eggs. The number of pigs excreting eggs and the mean EPG excretion continued to rise in the control group after day 45. In the treatment group the mean EPG excretion decreased significantly with 85.8 % from 6 days post treatment (day 51). Reductions in worm counts and EPG were calculated using both the arithmetic mean.
Conclusion: Pigfen® administered as a single dose at 5 mg per kg bodyweight eliminates very efficiently adult stages of *A. suum* and decreases the egg excretion with 85.8 %, already after 6 days. In opposite to some other anthelminthics, fenbendazole has also an ovicidal effect and kills both migrating and intestinal larval stages. Consequently a deworming strategy with Pigfen® - based on the prepatent period of 6 weeks - lowers the infection pressure in the environment rapidly.

Disclosure of Interest: None Declared

Keywords: Ascaris suum, Fenbendazole, Pigfen®

Parasitic Diseases

PO-PF3-141

EFFICACY AND UTILITY EVALUATION OF TOLTRAZURIL (BAYCOX® 5%) FOR COCCIDIOSIS IN PIGLETS

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Introduction: Toltrazuril is an anticoccidial drug commonly used in the treatment of coccidiosis in pigs. The objective of this study was to evaluate the long-term efficacy and utility of treatment of Toltrazuril (Baycox® 5%) for coccidiosis in piglets.
Materials and Methods: A field study was carried out in a farm of 4000 sows in the Emilia-Romagna region (Italy) where Toltrazuril had been used extensively for more than 10 years. According to the protocol in place, a single dose of 20 mg per kg body weight of active substance is administered to 3-5 days old piglets. The study lasted three months (May, June and July 2015) and consisted of three activities. The first activity was the systematic collection of the following data for each litter: a) the total number of pigs born, b) pigs born alive, c) stillborn pigs, d) weaned pigs, e) weight of pigs at weaning and f) the weekly number of litters with piglets of 10 days or older with enteric symptoms. The second activity was the routine collection of stool samples of piglets to be tested for the presence of oocysts of *Isospora suis* by McMaster microscopic technique. Finally, the third activity was addressed to piglets found dead after having shown enteric symptoms to establish the cause of death. For this last activity necropsy and laboratory testing were carried out.
Results: Data collected with the present study and those collected during the previous 5 years at the same farm don't present alterations compatible with coccidiosis outbreak. The total weight of litter weaned pigs collected during the study and those collected during the previous 5 years were not statistically different. On average, during the field study, the weekly number of litters with piglets of 10 days or older with enteric disease symptoms was 10 and this indicates that the incidence of suckling piglets with enteric problems was generally low at the farm. Oocysts of *Isospora suis* were not found in stool samples except for one case where fewer than 100 oocysts per gram were detected. Finally, lesions observed at necropsy and the following laboratory results suggested that infections of *Escherichia coli* and *Clostridium spp* were most likely the cause of death in piglets.
Conclusion: The results of this study suggest that the long-term use of Toltrazuril is effective in controlling coccidiosis in piglets.

Disclosure of Interest: None Declared

Keywords: Coccidiosis, Toltrazuril

Parasitic Diseases

PO-PF3-149

The effectiveness of an oral toltrazuril and iron combination in maintaining weaning weight by preventing coccidiosis and anaemia in piglets.

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Introduction: Coccidiosis occurs worldwide in association with intensive pig husbandry. In the intensive pig production piglets are supplemented with Iron to prevent iron deficiency anaemia. Traditional prevention of coccidiosis and iron deficiency anaemia has involved two separate intervention. Toltrazuril is well established product given orally to piglets in prepatent period to control coccidiosis and iron is supplemented traditionally by intramuscular route to young animals.
Materials and Methods: Effectiveness of an oral combination of toltrazuril and iron dextran (Baycox® Iron) to maintain weaning weight by preventing coccidiosis caused by *Isospora suis* and iron-deficiency anaemia in neonatal piglets was investigated on three commercial pig farms. Piglets were randomised within litter by bodyweight to treatment or control group. On SD 3 piglets allocated to the control group (CG) each received 1 mL Baycox®, containing 50mg/mL toltrazuril orally and commercially available iron (200 mg/piglet) by intramuscular injection. Piglets allocated to the treatment group (TG) each received 1 mL toltrazuril and iron combination orally (Baycox® Iron) containing 50 mg/mL toltrazuril and 228 mg iron as iron dextran. 6493 piglets completed the study.
Results: Bodyweight at weaning on SD 21 of piglets treated with the oral toltrazuril and iron combination was confirmed to be non-inferior to the control treatment with <1% difference between group mean body weights. Faecal samples from at least 10% of litters on SD 14 demonstrated control of coccidiosis. On SD 21 haemoglobin levels were above minimum levels to maintain health. There was no difference in mortality between the two groups. This large scale field evaluation clearly demonstrated the effectiveness of a combination of oral toltrazuril and iron (Baycox® Iron) in maintaining body weight at weaning compared to conventional treatment.
Conclusion: The combination was effective in preventing coccidiosis and anaemia and thus provides a valuable alternative that reduces stressful events in neonatal piglets. The combination product was safe without product related adverse events.

Disclosure of Interest: C. Bhushan Conflict with: Employee, K. Streyl Conflict with: Employee, J. Carlstron Conflict with: Employee, E. Dantas Conflict with: Employee, R. Mendoza Conflict with: Consultant, J. A. Torres Islas Conflict with: Employee

Keywords: None

Parasitic Diseases

PO-PF3-196

Large-scaled sarcoptic mange survey in slaughtered pigs in Northern Italy using a simplified and cost-effective monitoring technique

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Introduction: *Sarcoptes scabiei* var. *suis* is the etiologic agent of sarcoptic mange in swine. The diagnosis of mange, using the scraping from the skin and ears may be difficult in low level infestations. In these situations, a large number of pigs may have to be scraped to detect the presence of mange. The study aimed to describe an adapted method for a large-scale and cost-effective approach in the survey of sarcoptic mange in slaughtered pigs.
Materials and Methods: The sampling was carried out between August 2014 and January 2015 in 219 batches of 9-month-old slaughtered pigs belonging to 112 herds located in 20 different Italian provinces (6 regions: Emilia Romagna, Lombardia, Veneto, Piemonte, Marche and Friuli-Venezia Giulia). The sampling was performed on the slaughter line, during the regular slaughtering process, by skin scraping of the outer ear of at least 30 pigs per batch. The ears were not cut and removed from the carcasses.
Skin scrapings from the same batch were pooled and placed in a beaker with the addition of a 10% solution of Potassium Hydroxide (KOH). The samples were kept on a hotplate at about 70°, gently shaked until the complete clarification, transferred in a tube and centrifuged at 1500 rpm for 5 min. After the removal of supernatant the sediment was observed using the stereomicroscope. The result was expressed has positive or negative for sarcoptic mange. Out of the 112 herds included in the study, 68 herds were sampled once, 20 and 10 farms were sampled twice and three times respectively. Six farms were sampled between 7 and 10 times.
Results: A total of 30/219 batches were positive for *Sarcoptes scabiei*. The prevalence was 13,7% (IC 95%: 9.1% to 18.3%) with strong regional variation. Twenty two out of the 112 farms (19.6%; IC 95%: 12.2% to 27%) were positive for sarcoptic mange. No seasonal tendency could be detected. In the farms sampled several times, the positivity of different samplings was confirmed only in one case. The protocol used in this study despite 100% specific, could suffer of low sensitivity since the number of mites involved, is related to the clinical form of the disease. Nevertheless, pooling the samples within a batch offer a cost-effective approach to monitor a high number of farms and includes mange diagnostic as a routine practice in herd health monitoring.
Conclusion: The sarcoptic mange herd prevalence in Italy, using the method reported above, was estimated to be 19.6%. The approach described can be used during routine carcass processing for a continue monitoring of sarcoptic mange. This is of fundamental importance in particular for herds that have sub-clinical or mild clinical mange in their operations.

Disclosure of Interest: G. Maioli: None Declared, A. Scollo: None Declared, G. Leotti Conflict with: MERIAL SpA, F. Defilippo: None Declared, M. Veloci: None Declared, P. Bonilauri: None Declared, M. Dottori: None Declared, A. Luppi: None Declared

Keywords: Mange

Poster Abstracts

Parasitic Diseases

PO-PF3-234

Sensitivity to diminazene aceturate and isometamidium chloride of trypanosome isolates from pigs in Enugu North Senatorial Zone, Nigeria

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Introduction: The field control of animal trypanosomosis has, over the years, relied on two broad strategies: using chemotherapeutic agents on infected animals, and vector control. At present chemotherapy and chemoprophylaxis are the only practical methods available for the control of animal trypanosomosis, but their effectiveness is being threatened by a number of factors, which include increasing parasite resistance , treatment failures and unacceptable toxicity.
Materials and Methods: The sensitivity of trypanosome isolates from naturally infected pigs in Enugu North Senatorial Zone (18 isolates, comprising 16 *T. brucei* and 2 *T. congolense*) was evaluated in mice at two dose levels each of diminazene aceturate (7 and 28 mg/kg body weight) and isometamidium chloride (0.25 and 2 mg/kg) by the infection and treatment method.
Results: Multiple drug resistance was prevalent in the trypanosome isolates, as all 18 isolates (16 *T. brucei* and 2 *T. congolense*) tested were resistant to both diminazene aceturate (7 mg/kg b.w) and isometamidium chloride (0.25 mg/ kg b.w,), at the low dose levels tested. Sixteen of the isolates resisted the high dose levels of diminazene aceturate (28 mg/kg b.w), while six isolates were resistant to isometamidium chloride (2 mg/kg b.w).
Conclusion: It was concluded that trypanosome isolates from pigs in the study area exhibited resistance to both diminazene aceturate and isometamidium chloride, the two most commonly used trypanocides in the area. This phenomenon constitutes serious threat to chemotherapeutic control of porcine trypanosomosis in particular and animal trypanosomosis in general in Enugu North Senatorial Zone.

Disclosure of Interest: None Declared

Keywords: Diminazene aceturate, Isometamidium chloride, Sensitivity

Parasitic Diseases

PO-PF3-235

Clinical Isospora (syn. Cystoisospora) suis infection in fattening pigs during an outbreak of inclusion body rhinitis

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Introduction: *Isospora* (syn. *Cystoisospora*) *suis* is one of the most important causes of diarrhoea in suckling piglets worldwide and is associated with big economic losses. Usually, only suckling piglets < 3 weeks of age develop the typical lesions and signs of disease. Although *I. suis* is regarded as a primary pathogen, co-infections with bacterial or viral agents may be associated with more severe clinical signs and higher mortality rates. Porcine cytomegalovirus (PCMV, SuHV2) is extensively distributed in pig populations worldwide and is the cause of inclusion body rhinitis. In naive herds, PCMV causes disease characterized by reduced general condition, respiratory and neurological signs with increased fetal and piglet mortality; however, in older pigs the infection is often subclinical or mild and self-limiting.
Materials and Methods: In a Swiss pig herd (n= 9 sows, 30 growing pigs, 13 finishing pigs), a febrile disease characterized by anorexia, reduced general condition and almost 100% morbidity was first detected in finishing pigs before it extended to sows and growing pigs within a few days. Five growing pigs (age ~17 weeks) also had diarrhoea and after 3 weeks sneezing and dyspnoea were noted in 10 animals. Piglets delivered shortly before or during the outbreak (n=59) were stillborn (n=5), displayed respiratory signs (n=25), poor growth (n=14), lameness/stiff gait (n=4) or sudden death (n=10). One of the growing pigs showing diarrhoea was euthanized due to poor condition. Necropsy, histopathological, bacteriological, virological (Rotavirus A, TGEV, PEDV: immunochromatography; PCV-2: immunohistochemistry; SIV, PRV: real-time PCR; pADV, PCMV: PCR, sequencing) and parasitological (zinc chloride flotation) analyses were performed.
Results: A diagnosis of PCMV infection in the herd was achieved by histopathology (lesions and typical basophilic, intranuclear inclusion bodies in nose, liver, spleen, lung) and confirmed by PCR/sequencing on tissues and blood. Coproscopically, *I. suis* oocysts were detected in the faeces from fatteners with diarrhoea. Histopathologically, a severe fibrinopurulent jejunoileitis with extensive atrophy and fusion of intestinal villi, loss of goblet cells and crypt abscesses were observed in the euthanized fattener. Abundant *I. suis* stages (mainly typical merozoite pairs within enterocytes) were associated with these severe lesions.
Conclusion: While *I. suis* generally produces clinical disease only in suckling piglets, it was surprisingly associated with severe intestinal lesions and diarrhoea in a 17 week-old fattening pig co-infected with PCMV. The interaction mechanisms between these two pathogens are, however, still unknown.

Disclosure of Interest: None Declared

Keywords: fattening pigs, Isospora suis, Porcine Cytomegalovirus

Parasitic Diseases

PO-PF3-270

PREVALENCE OF PARASITIC HELMINTHES IN SLAUGHTERED PIGS IN IBADAN NIGERIA

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Introduction: Documentation of parasitic disease prevalence in many developed countries has been well articulated but very little of such documentation is on record in swine slaughtered in abattoirs in most developing countries including Nigeria. The impact of parasitic helminthes on pig health, human health (especially to pork consumers), and economies of the swine industry in Nigeria needs to be properly documented. During routine postmortem examination of slaughtered pigs at the Bodija abattoir in Ibadan, Southwestern Nigeria, faecal samples collected were examined and helminthes examined in the pigs are documented in this study.
Materials and Methods: 397 faecal samples were collected from different breeds of pigs. Age, sex and breeds of the pigs were determined. The faecal samples on ice pack were transported to the laboratory of the parasitology unit in the University of Ibadan for faecal egg analyses using flotation technique and quantitative analysis using McMaster's technique.
Results: Out of the 397 pigs examined, 294 (74%) were infected with four identified helminthes parasites. The helminthes species in the infected pigs were *Ascaris suum (36%), Trichuris suis (7%), Hyostrongylus rubidus (22%), Oesophagostomum dentatus (42%).* Exotic breeds constituted a higher percentage of the slaughtered pigs (75.57%), however, local breeds (100%) were more infected with helminthes parasites. Of the 294 infected pigs, 64.63% were males and 35.37% females. Adult constituted 62.95% and young 37.09% of the infected pigs.
Conclusion: This study documented four helminthes of economic importance (*Ascaris suum, Trichuris suis, Hyostrongylus rubidus* and *Oesophagostomum dentatum*), of these three (Ascaris suum, Trichuris suis and Oesophagostomum dentatum) of public health importance. There is therefore the need for management interventions in pig industry to control helminthes infection.

Disclosure of Interest: None Declared

Keywords: Nigeria, Public health importance, Swine helminthes

Parasitic Diseases

PO-PF3-292

Trichomonads and Brachyspira murdochii infection in pigs with colitis

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Introduction: Gastrointestinal disease is one of the leading causes of clinical signs in growing and finishing pigs. We herein describe several diagnostic cases of finisher pig colitis wherein both novel and common pathogens were discovered.
Materials and Methods: Necropsies of 5 pigs, 10-13 weeks of age, with diarrhea and weight loss, were performed at two swine farms, each housing >15,000 growing pigs. Tissue samples including colon were submitted to the UMN-VDL between 12/19/2014 and 1/9/2015.
Results: Histologic examination of the colon in all cases revealed moderate (cases 1-4) to mild (case 5) proliferative colitis with intralesional trichomonads. *Brachyspira murdochii* was isolated from the colon and detected by immunohistochemistry in cases 3-5, but not in cases 1-2. Positive immunoreactivity for *Trichomonas* spp. in sections of colon was observed in cases 1-2 by immunohistochemistry, performed at UC-Davis. *Trichomonas* spp. were present in the lumen of dilated and hyperplastic mucosal glands, invading the lamina propria, and rarely in the cytoplasm of goblet cells and enterocytes. Trichomonads were also found within the cytoplasm of mucosal gland and proprial macrophages (phagocytosis), suggesting elicitation of an innate immune response.
Conclusion: Common causes of colitis in pigs also include *Lawsonia intracellularis* and *Salmonella enterica;* however, they were not detected in these 5 index cases. Interestingly, *L. intracellularis* and/or *Salmonella* spp. were detected in the colons of several pigs submitted subsequently from both farms (data not shown).
Lesions in these pigs were consistent with an infectious colitis. Trichomonads infection with or without *Brachyspira murdochii* co-infection was diagnosed in all cases. Both agents have been considered facultative enteric pathogens in swine. Whether this co-infection was the inciting factor for the colitis is controversial. We speculate that the severity of colonic lesions induced is greater when both pathogens are present.

Disclosure of Interest: None Declared

Keywords: colitis, diarrhea, trichomonads

Poster Abstracts

Reproduction

PO-PW1-209

INFLUENCE OF LIANOL ON REPRODUCTIVE PERFORMANCE AND LITTER SIZE OF SOWS DURING PERIODS OF HEAT STRESS IN ITALY.

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Introduction: Sow litter size and oestrus behaviour are highly susceptible to heat stress. One of the causes of this reduction in performance is a state of negative energy balance (NEB) during the lactation period due to reduced feed intake. Literature describes that IGF-1 (insulin-like growth factor 1) is a likely candidate to mediate this effect of NEB on reproduction and is clearly involved in ovarian physiology and folliculogenesis. High levels of IGF-1 have beneficial effects on reproduction and litter size in sows. Former research has demonstrated a positive effect of Lianol®, a complementary feed based on Fermented potato protein (FPP), on plasma IGF-I levels in sows. This study focusses on the effect of supplementing Lianol® Ferti to sows on the reproductive performance and litter size during high environmental temperatures.
Materials and Methods: At a commercial Italian sow farm, 2 consecutive production groups were followed and each production groups was divided in a control group (n=75) and a treatment group (n=73) were compared. Both groups had an equal parity distribution, body and condition and were held under identical conditions (housing, diets,…).In the treatment group, the sows received from 3 days before till 3 days after weaning 1 Lianol® Ferti tablet. And the gilts received 1 Lianol® Ferti tablet for 5 days starting after the synchronization treatment. To see the effect of Lianol® on parity and body condition at entry of farrowing room, sub-classes were created according back fat (slim, ≤ 17 mm – normal, 18-19 mm – fat, ≥20 mm) and parity (primiparous - 1-2 parity - ≥ 3 parity).
The interval weaning-pregnancy, pregnancy rate (with ultrasound scan) and number of sows that returned in estrus were calculated. At farrowing, the number of live born, stillborn and mummified piglets was recorded
Results: This trial showed a tendency in improved weaning-pregnancy interval (minus 2.9 days, p=0.08) and number of sows that returned in estrus (minus 3.9 %, p=0.08) for the treatment group. For the interval weaning-pregnancy, these improvements were significant for the sub classes: normal back fat sows (minus 6.78 days, p=0.05) and *1-2 parity* sows (minus 5.88 days, p=0.05). The treatment group, had a significant increase in total born piglets (plus 1,3 piglets, p=0.026) .
Conclusion: Supplying Lianol® Ferti during hot temperatures to sows significantly increased the number of total born piglets and improved the reproduction performance. Lianol® Ferti could be useful on sow farms suffering from heat stress.

Disclosure of Interest: None Declared

Keywords: heat stress, Sow Performance, summer infertility

Reproduction

PO-PW1-227

Effect of altering ratio of gonadotropins on reproductive performance of primiparous sows during the seasonal infertility period

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Introduction: A major problem influencing pig production is failure to meet breeding targets due to sows having prolonged wean-oestrus intervals or anoestrus. Normally, oestrus can be induced in weaned sows by injection of 600 to 1,000 IU eCG or a combination of 400 IU eCG and 200 IU hCG (PG600) on the day of weaning. However, we found increased failure to respond to PG600 treatment in some periods of the year, such as in the hotter months. We suggest this may be due to the 200 IU hCG in PG600 providing an inadequate duration of LH-like activity and that an increased duration of LH-like activity would enhance ovarian follicular development and associated rates of ovulation. Therefore, we hypothesis that fertility of weaned sows receiving PG600 in the seasonal infertility period will be improved by injection of supplemental hCG. The aim of this study was to investigate the effect of gonadotropins and their ratios on fertility of primiparous sows in the seasonal infertility period.
Materials and Methods: During the seasonal infertility period 150 Landrace x Yorkshire primiparous sows were assigned to be control (n=50), Gn600 (n=50) or Gn800 (n=50). At weaning sows in Gn600 and Gn800 groups were given (IM) 400 IU eCG plus 200 IU hCG or 400 IU eCG plus 400 IU hCG, respectively; controls received no treatment. Oestrus stimulation with fenceline boar contact was performed from 2 d after gonadotropin injection and wean-to-oestrus intervals recorded. Sows exhibiting oestrus were investigated for the number of pre-ovulatory follicles (>0.6 cm) by ultrasonography. Sows not exhibiting oestrus by 14 d after gonadotropin injection were culled. All oestrous sows were inseminated at least twice and subsequent farrowing rates and litter sizes at birth recorded.
Results: We found 90% of Gn800 sows returned to estrus within 7 d in comparison with 42% of Gn600 and 34% of controls (p≤0.05). Gn800 sows had shorter WOI than Gn600 and control group (5.5±1.6 vs.7.7±2.3 and 8.5±3.2, respectively; p≤0.05). Compared to controls, Gn800 and Gn600 sows had more preovulatory follicles (17.2±1.7 vs. 20.1±1.5 and 19.0±0.9, respectively) and higher farrowing rates (85% vs. 95% and 93%, respectively) (p≤0.05). There were no treatment differences in litter size.
Conclusion: Modified exogenous gonadotropin treatment was able to significantly enhance oestrus expression in primiparous sows and also improved number of preovulatory follicles and farrowing rate.

Disclosure of Interest: None Declared

Keywords: gonadotropins, infertility period, primiparous sows

Reproduction

PO-PW1-238

Relationship between semen parameters and meat quality characteristics in Piétrain boars

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Introduction: In several countries, Piétrain boars are indispensable as a terminal sire and constitute a large group of boars kept by the AI centers. Selection of these boars is mainly based on fattening performance and meat quality characteristics. However, the effects of this focused selection strategy on semen quality have been poorly investigated. The main objective of this study was to assess the semen quality of Piétrain boars originating from different AI centers and to correlate the results with the carcass quality characteristics of these boars. Additionally, the storability of the semen doses during the 5 successive days of storage was investigated.
Materials and Methods: Freshly diluted semen doses from 147 boars (age: 6.5 to 87.5 months) originating from 10 artificial insemination (AI) centers were used and stored for five days at 17°C. Motility was assessed daily with CASA (Hamilton-Thorne), morphology and concentration were assessed on day of semen collection (D0) by eosin-nigrosin staining and the Bürker counting chamber, respectively. The above mentioned data were correlated with the lean meat percentage, loin eye depth and backfat thickness using linear mixed models taking into account the clustering of boars within AI centers and the repeated measurements for each semen dose.
Results: The 5-day average motility (±SD) across all AI centers was 76.3±12.4%. Average values (±SD) for morphology and concentration on D0 were: live spermatozoa 91.0±4.9%, live normal spermatozoa 83.2±8.4%, and concentration 28.9±10.7 ($x10^6$ spermatozoa/ml). Backfat thickness (cm) and loin eye depth were significantly associated ($p<0.05$) with both motility and progressive motility. Boars having more backfat showed higher semen motility from D0-2 (1.16% of extra motility for every 1 cm backfat), while backfat was negatively related to motility during D3-4, indicating a more pronounced negative impact of longer storage duration on semen motility of boars with more backfat. Backfat thickness was positively associated with progressive motility from D0-4. Loin eye depth was positively associated with motility and progressive motility from D1-4 ($p<0.05$). Lean meat percentage was not found to be significantly associated with motility and progressive motility ($p>0.05$). None of the carcass quality characteristics were significantly associated with semen morphology and concentration.
Conclusion: Boar studs could maximize the potential of creating Piétrain sire lines that combine good productivity with high fertility by avoiding a strict selection based on reduced backfat, and by including in the selection criteria the loin eye depth. Additionally, the expiry dates provided by some AI centers should be revised.

Disclosure of Interest: None Declared

Keywords: Boar, Carcass quality, Semen quality

Reproduction

PO-PW1-253

Genetic Characterization of ORF5 and ORF7 from an atypical PRRSV case in 2014 in Malaysia.

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) is one of the most expensive diseases of modern swine production & results in annual economic losses and cost the industry over 600 million USD in U.S alone and billions of dollars worldwide. An atypical PRRS case was observed in early 2014 characterized by late-term abortion, fever and sudden increase in sow mortality which dragged on for over 6 months.
Materials and Methods: Sampling
Lungs and lymph nodes were collected from the farm for disease investigation.
Phylogenetic analysis of the ORF7, ORF5 and nsp2
Sequencing of the ORF5, ORF7 & nsp2 gene of PRRSV was done using the BigDye Terminator v3.1 cycle sequencing kit chemistry. The phylogenetic tree was constructed by using the Maximum Likelihood method, generated by Mega 6®.
Results: The extraordinary genetic variation of RNA viruses poses a challenge for diagnosis. After various attempts and optimization, nsp2 gene was not successfully amplified. Analysis of the ORF7 showed that the sequence had 98.3% nucleotide and 97.5% amino acid sequence similarity to vaccine strain VR-2332, RespPRRSV and other mutant/chimeric virus strains from South Korea, China, Denmark & USA. Analysis of the ORF5 gene showed that the gene was also 99.3% genetically similar and closely related to a modified live vaccine strain, RespPRRSV and both genes phylogenetically clustered with these vaccine and mutant/chimeric strains.
Conclusion: Rapid evolution due to high mutation rate has led to new generations of genetically and antigenically variable virus strains in the field. We were unable to sequence the nsp2 gene in this study. Nsp2 is a multi-domain protein and studies have reported that the presence of the nsp2 protein as different isoforms in PRRSV-infected cells, which appear to share the same N terminus but differ in their respective C-termini suggest that it is highly heterogeneous making it tricky to detect the virus with publically available published primers. More so, vaccine strains or derivatives of vaccine strains may also induce disease in the field and persist in vaccinated pigs and spread to non-vaccinated pigs contributing to PRRSV virulence in the field and the inability for effective vaccination in the field. The results from this study suggest that the virus that persisted in this farm is a product of a recombination event between vaccine strains and field isolates which has developed into a mutant/chimeric strain. Genetic and evolutionary analyses of full length genomes are important to delineate the degree of homology among PRRSVs and for effective vaccine design in future.
Acknowledgement
The authors would like to thank Prof. Dr. Henry Too and Dr. Francois Joisel for their advice & contribution.

Disclosure of Interest: None Declared

Keywords: Porcine reproductive and respiratory syndrome virus (PRRSV)

Poster Abstracts

Reproduction

PO-PW1-216

Influence of immunological suppression of gonadotropin releasing factor (GnRF) on carcass characteristics and cutting yields of heavy weight gilts

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Introduction: The objective of this study was to determine the effects of a GnRF vaccine during the late finishing phase of heavy weight gilts on carcass traits and pork cutting yields. Management of market gilts with Vivax (Zoetis, São Paulo, Brazil) leads to production of antibodies against GnRF, which suppresses estrus. Considering that estrus is a period of markedly reduced growth and feed intake potentials, immunized gilts can have increased growth performance. Enhancing growth and intake potential in animals can produce carcasses with better quality, weigh, and cutting yields.
Materials and Methods: Gilts were initially weighed and allotted to a pen (n=72; 2 pigs/ pen) based on BW in a completely randomized design. The treatment group received the first anti-GnRF vaccine dose at 15 wk of age (V1) and the second dose at 19 wk of age (V2), while the control group received two injections of saline. Daily boar exposure (DBE) occurred from 21 to 25 wk of age, and the animals were slaughtered at 25 wk of age (S) (6 wks after second dose). Seventy two hot carcasses were evaluated for back-fat thickness (mm), lean meat yield (%), weight (kg), and loin depth (mm). Pork cutting yields were analyzed on the dissected carcasses of the 12 gilts closest to pen average ending live weight in 18 of the 36 pens. Data were submitted to ANOVA and means were compared by F-test.
Results: Immunization against GnRF did not showed any differences in back-fat thickness (P = 0.21239), lean meat yield (NS), weight (NS), and loin depth (NS). The treated group had 4.57% greater cold carcass weight (P < 0.01) and 11.17% greater gross pig flank weight (P < 0.001) compared with the control group. Managing gilts with an anti-GnRF vaccine did not impact weight of shoulder (NS), loin (P = 0.10395), belly (P=0.11976), or ham (NS), and did not influence belly thickness (NS), ham's meat (NS), skin plus fat (NS), or bones yield (NS).
Conclusion: Immunological suppression of GnRF improved cold carcass and gross flank weights of treated gilts compared with the control group. Further studies are needed to assess possible unknown effects of immunocastration on pork cutting yields.

Disclosure of Interest: None Declared

Keywords: Gilts, Pork carcass, Vivax

Reproduction

PO-PCO1-015

COMPARISON OF REPRODUCTIVE PERFORMANCE OF FIXED TIME INSEMINATION VERSUS CONVENTIONAL MULTIPLE INSEMINATION PROTOCOL IN A COMMERCIAL FARM

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Introduction: It is well known that to ensure best fertility and prolificacy results, sows need to be inseminated at least 24h before ovulation. As exact timing of ovulation is unknown, conventional reproductive management with multiple inseminations is usually implemented to optimize reproductive data. Consequently, farmers invest a lot of time in heat detection and insemination. MSD AH has developed a single Fixed Time Insemination program (FTI) using Porceptal® (Buserelin 4µg/ml, a GnRH agonist), that allows for a more efficient workforce and a reduction in semen doses. The aim of this trial was to demonstrate that FTI gives equivalent technical reproductive results compared with classical breeding programs.
Materials and Methods: The trial was conducted in a 950 sow farm in Spain. A total of 200 sows were randomly allocated into two groups based on production cycle: 110 sows in Control Group (C) and 90 sows in Porceptal Group (P). From weaning to Porceptal® injection, all sows were managed the same. Control sows were inseminated following the standard farm insemination protocol. Porceptal® sows were injected with 2.5ml Porceptal® i.m 88-89h post-weaning. About 31-32 hours later, sows were checked for heat, and if positive, were inseminated with one dose of commercial semen. No additional heat detection or insemination was done after this. In both groups post-cervical artificial insemination was applied. Reproductive data were compared via Pearson Chi Square Test, Levene Test and ANOVA.
Results: Percentage of sows with post-weaning estrus was the same in both groups (P 93,3% vs C 90,9%; p=0,7) and fertility was also not different (P 92,7% vs C 90%; p=0,8). Gestation length was one day less in P Group (P 113,7 vs C 114,7). Prolificacy was comparable in both study groups (P 13,54 vs C 13,46 piglets; p=0,7). Average stillbirths was 0,25 piglet lower in Porceptal Group than in Control (P 0,55 vs C 0,8). With respect to farrowing day, 75% of the farrowings in the P group occurred between Wednesday and Friday, which was 7,6% more than in C group. One sperm dose per sow was used in the P Group, compared to an average of 2.5 doses per sow in the Control group.
Conclusion: No significant differences in reproductive parameters were found between animals following a single FTI or a conventional multiple insemination program. Additionally, FTI has clear advantages because it simplifies estrus management, concentrates farrowings, reduces stillbirths and semen doses. An economical study based on stillbirths, workflow and reduced semen doses supported a ROI of 2,6 for the P group, making FTI an efficacious and profitable alternative.

Disclosure of Interest: None Declared

Keywords: FTI, Porceptal, ROI

Reproduction

PO-PW1-232

Does duration of farrowing affect plasma oxytocin concentrations of sows at the subsequent oestrus?

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Introduction: Previous studies showed that prolonged farrowing could lead to decrease in fertility rate at the subsequent oestrus in sows. Oxytocin is well-known for its role in myometrial activity and reproduction process in oestrous sows. We, therefore, investigated whether duration of farrowing affects plasma oxytocin concentrations of sows at subsequent oestrus.
Materials and Methods: A total of 31 crossbred sows (Finnish Yorkshire × Finnish Landrace) were allocated two treatment groups according to duration of farrowing: 1) SHORT (159 ± 29 min): 14 sows (parity 3.8 ± 2.6) with shorter than 200 min farrowing duration, and 2) LONG (579 ± 263 min): 17 sows (parity 5.1 ± 2.5) with longer than 300 min farrowing duration. Duration of farrowing was determined by the birth interval between the first and the last piglets. After a 4 week lactation, the sows were confined in the stalls and catheterized three days before the expected oestrus through the auricular vein following a nonsurgical catheterization procedure. Upon triggering the standing reflex by boar introduction, blood samples were collected -15, -10, -5, 0, 1, 2, 3, 4, 6, 8, 10, 15, 20, 25, 30, 40, 50 and 60 minutes while the boar introduction occurred at 0 min. Plasma oxytocin concentrations were measured using ELISA kits (Enzo Life Sciences, Switzerland). Repeated measures using mixed model were used to analyze periodical oxytocin concentrations according to the boar introduction.
Results: Plasma oxytocin concentrations of oestrous sows in the LONG group (22.2 ± 1.8 pg/ml) were greater than in the SHORT group (15.6 ± 2.0 pg/ml) during the whole sampling period, i.e. 15 min prior to until 60 min post boar introduction ($P < 0.05$). During the presence of the boar for 10 min, oestrous sows in the LONG group (27.8 ± 3.6 pg/ml) tended to have greater plasma oxytocin concentrations than in the SHORT group (17.8 ± 4.0 pg/ml; $P = 0.07$).
Conclusion: The results indicate that prolonged farrowing might lead to an increase in plasma oxytocin concentrations of oestrous sows at the following breeding. However, further research is needed to demonstrate interrelationship between duration of farrowing, oxytocin concentrations during estrus, and follicle development in sows.

Disclosure of Interest: None Declared

Keywords: Farrowing time, Oxytocin, Sow

Reproduction

PO-PW1-256

A CASE REPORT OF ATYPICAL PORCINE REPRODUCTIVE & RESPIRATORY SYNDROME IN MALAYSIA PIG FARM.

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) is endemic in most of the swine-producing countries, and majority controlled with PRRS MLV vaccine. The concern over PRRS MLV vaccine arose as increase of atypical PRRS outbreak, although many of the affected had been vaccinated multiple times with PRRS MLV vaccine. This is the first reported case of atypical PRRS outbreak in a Malaysian pig farm.
Materials and Methods: History and clinical signs
In February 2014, a farrow-finish 300 sow herd reported an outbreak of late-term abortion, 30% of repeat to estrus, and more than 50% of pre-weaning mortality. Sow herd had been regularly vaccinated with Aujeszky's vaccine, swine fever and PRRS MLV. Mortality of grower & finisher were increase with porcine respiratory disease complex. One week after a schedule blanket vaccination in sow herd with PRRS MLV, there were more than 50% of gilt and first parity sow were sudden died near to term with hyperemia and pyrexic, and 20% of late-term abortion. Umbilical haemorrhage was observed in some cases. Piglet showed ill thrift with periocular oedema and conjunctivitis. Weaners exhibited dyspnea and lethargy and mortality rose to more than 80%.

Autopsy findings
Lungs were mottled and fail to collapse, some with interstitial pneumonia. Lymph nodes, especially mediastinal lymph nodes were enlarged, with or without haemorrhagic.

Molecular findings
Lungs and lymph nodes were collected for molecular study and phylogenetic analysis of ORF5, ORF7 and nsp2 gene. Sequencing was done using the BigDye Terminator v3.1 cycle sequencing kit chemistry. The phylogenetic tree was constructed by using the Maximum Likelihood method, generated by Mega 6®.
Results: RT-PCR showed that it was positive for PRRSV US strain. Analysis of the ORF7 showed that the sequence had 98.3% nucleotide 97.5% amino acid sequence similarity to vaccine strain VR-2332,and RespPRRSV. Analysis of the ORF5 gene showed that the gene was also 99.3% genetically similar and closely related to a modified live vaccine strain, RespPRRSV.
Conclusion: The clinical & phylogenetic findings highly suggest that vaccine-derived virus may contribute to this atypical PRRS outbreak.

Acknowledgement
The authors would like to thank Prof. Dr. Henry Too and Dr. Francois Joisel for their invaluable advice & contribution.

Disclosure of Interest: None Declared

Keywords: Porcine reproductive and respiratory syndrome virus (PRRSV)

Poster Abstracts

Reproduction

PO-PW1-219

The impact of birth weight on spermatogenesis in boars

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Introduction: Low birth weight piglets are a reality in commercial farms and have been associated with functional disorders of several organs systems, resulting in deleterious consequences during postnatal life. There is strong evidence that low birth weight pigs present compromised postnatal growth and performance and poor meat quality. However, reports of birth weight effects on the reproductive system are scarce, especially in boars. Therefore, the aim of the present study was to evaluate testicular parameters associated with spermstogenesis in different birth weight boars.
Materials and Methods: Forty-eight newborn male pigs Agroceres-PIC genotype (crossbred Landrace, Large White and Duroc) from 24 litters were selected immediately after birth, and identified as falling into two birth weight categories: high (HW: range 1.85 to 2.15 kg; n=24) and low (LW: 0.85 to 1.15 kg; n=24) littermates. A sub-set of 12 pairs of male littermates from each experimental group was orchiectomized at eight days post-partum and another sub-set of 12 pairs of male littermates, orchiectomized at eight months of age. Testicular samples were collected and subjected to different preparations according to the histomorphometrical and sperm head count analysis. Data were analyzed as a randomized complete block design and the comparison between means was performed by t-test.
Results: HW boars had greater body and testicular weights than LW boars at both ages ($P < 0.05$). Overall, birth weight affected somatic and germ cells numbers in the neonatal testis and spermatids numbers in the post-pubertal testis, as shown by higher number of those cells in HW boars ($P < 0.05$). LW was not associated with depletion in spermatogenesis efficiency, represented by mitotic, meiotic and Sertoli cell efficiency indexes. Further evidence of normal spermatogenesis efficiency was established by counting type A spermatogonia and preleptotene spermatocyte per seminiferous tubule cross section, which were similar between both experimental groups. However, a significant reduction in the number of pachytene spermatocyte and round spermatid was observed in LW boars ($P < 0.05$), that caused a decrease in the total number of round spermatids ($P < 0.05$). These differences were proportional to testis size and may not be associated with impaired testicular function.
Conclusion: These findings suggest that prenatal programming of testis development will predetermine the reported relationship between adult testis size and lifetime semen production. Therefore, additional studies are necessary to better understand the effects of birth weight on other reproductive parameters related to semen quality and fertility.

Disclosure of Interest: None Declared

Keywords: Birth weight, boar, spermatogenesis

Reproduction

PO-PW1-230

Fixed time insemination (FTI) in sows: a controlled field study

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Introduction: An effective insemination management is the basis for a successful piglet production. Hormonal stimulation and synchronization is common practice in farms, mostly using equine chorionic gonadotropin (eCG). A FTI protocol is now available using Buserelin (Porceptal®, MSD Animal Health), a synthetic GnRH-analog compound. The main feature of this protocol is the requirement for only one insemination per sow. This study was conducted to compare the FTI with a toleration oriented approach using eCG for stimulation.
Materials and Methods: The farm houses 600 sows (DanAVI) in Northwestern Germany. It operates in a 4 weeks cycle with a suckling period of 21 days. The herd had no signs of any reproductive disorder in the last years. Sows are weaned and moved to the service center on Wednesdays. Starting from Sunday the sows have boar contact twice a day. One group of sows was divided into two groups: Porceptal 1 (P1, 52 animals) and Control 1 (C1, 62 animals). The same applied to another group six weeks later (P2, 46 sows and C2, 71 sows). The inclusion was not randomized, as groups P1 and P2 consisted of sows with 3 or more farrowings (mean 4.21 and 4.89), whereas groups C1 and C2 included all gilts and younger sows (mean farrowings 1.97 and 2.62). Groups P were injected with 2.5 ml Buserelin (Porceptal®, MSD Animal Health) 85h after weaning; groups C were injected with 800 IU eCG (Pregmagon®, IDT-Biologika, Germany) 24h after weaning. Sows treated with Buserelin were inseminated once 33h after the injection. Control animals were inseminated 2-3 times 16-18 h apart, depending on their toleration.
Results: The reproductive performance showed no significant difference. Regarding the farrowing quota P1 and P2 had 90% and 83.93%; C1 and C2 had 89.2% and 94.37%, respectively. Mean piglets born alive in the P groups combined were 13.4, in the C groups 13.5. For weaned piglets, the groups P showed a mean of 12.4, the control a mean of 12.5. Other traits, like piglet losses, mummies and the variation of farrowing dates did also not differ significantly between groups.
Conclusion: A FTI protocol using Buserelin resulted in an equivalent reproductive performance, compared to standard protocols using eCG. However, the trial was conducted on a well-managed farm with no history of reproductive failure and on this farm focused on the experienced sows. Here the FTI yielded equivalent results in all important reproductive traits. This management tool can improve the performance in respect for costs and workload of healthy and well managed herds. Further investigations should be conducted regarding the use in younger sows and on farms with reproductive problems.

Disclosure of Interest: None Declared

Keywords: Buserelin, FTI, reproduction

Reproduction

PO-PCO1-005

Teat necrosis of newborn piglets influenced by farrowing induction and boar effect

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Introduction: Teat necrosis of newborn piglets might result in shorter teats, 'pin teats', teat sphincter insufficiency, or non-functional, 'blind' teats in affected female piglets. It can cause substantial economic loss in the production of high-quality breeding gilts. Protective methods (adhesive bandages) are rarely used in the practice or, even if applied, sometimes their use is belated or not effective enough. Thus the knowledge of prevalence-influencing factors is crucial in order to minimize losses. Therefore, the aim of our investigation was to determine the effect of farrowing induction and boar on the prevalence of teat necrosis.
Materials and Methods: The investigation was carried out in a farrow-to-finish farm working with 900 sows. The pregnant gilts and sows that did not farrow by the 115th day of pregnancy were administered 2 ml PGF Veyx injection (Veyx-Pharma, Germany) i.m.. No teat protection was used on the piglets during the trial. The teats of piglets were observed at the age of 3–4 days. Statistical analyses were carried out with SAS (SAS Institute Inc., Cary, NC, USA) statistical software using the GLM procedure. Litter size at birth was used as a covariate factor in the analyses of treatment effects. In case of significant treatment effects the statistical difference between means was determined by Tukey's test.
Results: The litter size of sows with induced (n=87) or natural farrowing (n=112) was similar (11.3 vs. 11.5; P>0.05). However, in litters of sows with induced farrowing the average number of piglets having teat necrosis strongly tended to be higher (6.1 vs. 5.4, P=0.056). It is generally accepted that sows producing elevated levels of oestrogen during farrowing deliver piglets born with swollen teats. If the floor surface is not smooth enough, it can traumatise the teats, resulting in necrosis. Recent research indicates that the application of PGF2α analogues can further increase the sow's oestrogen level. This supports our findings and the generally accepted theory regarding the processes underlying teat necrosis. In our study, certain boars had significantly (P of boar effect < 0.001) more affected piglets than other boars of the same genotype. This phenomenon is quite interesting as it suggests that some boars transmit to their progeny an unknown factor that increases the sensitivity of piglets to elevated oestrogen in sows.
Conclusion: The endogenous oestrogen effect has been associated with elevated oestrogen levels of sows at farrowing, which is even more pronounced if induced farrowing is practised.
Selection of boars for a low prevalence of teat necrosis among their progeny may be a natural way to reduce predisposition to teat necrosis.

Disclosure of Interest: None Declared

Keywords: piglets, teat necrosis, oestrogen

Reproduction

PO-PW1-223

THE INFLUENCE OF SUPPLEMENTING LIANOL TO A FLUSHING DIET ON THE REPRODUCTIVE PERFORMANCE OF A CANADIAN SOW FARM.

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Introduction: Previous research demonstrated a positive effect of Lianol® Solapro, a complementary feed based on fermented potato protein, on plasma insulin-like growth factor-1 (IGF-1) levels in pigs. Negative energy balance (NEB) of sows in lactation can influence reproduction performance. Literature describes that IGF-1 (insulin-like growth factor 1) is a likely candidate to mediate this effect of NEB on reproduction and is clearly involved in ovarian physiology and folliculogenesis. High levels of IGF-1 have beneficial effects on reproduction and litter size in sows. Former research has demonstrated a positive effect of Lianol® on plasma IGF-I levels in sows. The objective of this study was to evaluate the effect of this complementary feed stuff on reproduction performance and litter size in sows when given after weaning.
Materials and Methods: This research was conducted on a research farm in Canada. Based on parity (average 4.14) and body condition, 102 hybrid sows origination from 1 production group were equally divided in a control group (n=52) and treatment group (n=50). All the animals were housed under equal conditions and fed the same diets. The treatment group received from weaning till 3 days after onset of oestrus Lianol® Solapro. This was added as a top dressing at a rate of 2.5 kg/ mT. Reproduction parameters like wean-to-oestrus interval, insemination rate as the number of piglets (total born, live born, still born, and mummified) in the following farrow were recorded.
Results: This trial showed a significant increase in total born and live born piglets between the treatment group and control group with respectively 1.06 piglets (p=0.04) and 1.13 piglets (p=0.04). The treatment group recorded a higher pre-weaning mortality but total number of weaned piglets was higher (0.6 piglets). For the parameters wean-to-estrus interval, pregnancy rate and returner, no significant differences were detected between both groups. Both effects were more pronounced in multiparous sows (data not shown).
Conclusion: Supplying Lianol® Solapro to the sow for 1 week after weaning (example in the flushing feed) significantly increased the number of total and live born piglets. Adding Lianol® Solapro just after weaning could be an effective strategy to improve sows performance.

Disclosure of Interest: None Declared

Keywords: fertility, litter size, sow reproduction

Poster Abstracts

Reproduction

PO-PW1-254

Bilateral scrotal swelling in a boar

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Introduction: A 3-year-old Landrace boar with a bilateral swelling of the scrotum was submitted to the Swine clinic in August. History reported a period of hyperthermia, anorexia, depression and weight loss in May. Recovery was obtained after a treatment based on antibiotic (lincomycin and ceftiofur) and anti-inflammatory (steroid) medication injections. On July, the boar fell down after a supervised natural mating.
Materials and Methods: The present poster describes our clinical approach and conclusions.
Results: The boar had a good general condition and a normal appetite. Scrotal palpation was painless. The left hemiscrotum was more enlarged than the right one. Serological analysis allowed to exclude Aujeszky disease virus, Brucella suis and Chlamydia spp., the main agents responsible for orchitis in pigs. Ultrasonography revealed multiple hypoechogenic cavities around both testicles. Semen analysis performed using microscope showed azoospermia. Dissection of the scrotal tissues - performed in October - revealed the presence of kystic structures filled with serous fluid; the largest one had a diameter of approximately 15 cm. These kystic structures were surrounded by an abondant fibrous connective tissue. Histological examination showed the presence of large amount of lymphocytes and hemosiderophages around the kysts. No sign of spermatogenesis was detected in testis. An hyperplasia of the interstitial tissue in detriment to seminiferous tubules was also observed.
Conclusion: These results led us to confirm the traumatic origin of the bilateral scrotal swelling observed in this boar. The lesions were the result of a partial resorption of a complex extratesticular hematoma.

Disclosure of Interest: None Declared

Keywords: boar, reproduction, scrotal enlargement

Reproduction

PO-PW1-224

The serum leptin concentration and condition of gilts and sows

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Introduction: Leptin is the hormone produced in the adipocytes of white adipose tissue. Concentration of leptin in blood serum is correlated with thickness of backfat. It was confirmed that sows with higher leptin concentration show heat faster after weaning. To properly choose sows for mating, it is necessary to determine their body condition. It could be done using BCS (subjective method) or USG (objective method). In the literature breeding condition is described as not too thin and not too fat. It is expected that sows in poorer condition after weaning should have lower concentration of leptin what is undesirable because low concentration of leptin decrease AI efficiency. The aim of the study was to compare results of objective and subjective body condition estimation with leptin concentration on AI day.
Materials and Methods: The study was carried out on 180 sows (29 gilts and 151 multiparous) in industrial farm. Animals were estimated on insemination day - BCS and backfat fat at P2 point, 30 minutes before AI blood samples were collected to measure leptin concentration.
Sows and gilts were assigned to three groups, depending on backfat thickness at P2: Gilts group I (n=10) < 16 mm, group II (n=5) between 17-20 mm and group III (n=2)>20 mm; Sows group I (n=14) <18 mm, group II (n=15)19-22 mm, group III (n=10) >22mm. Reproduction performance was estimated basis on: insemination efficiency, no of piglets born (alive, stillborn, mummies). Statistical analysis were made by the use of Anova, differences between analyzed groups were determined by Duncan test.
Results: According to the USG measurement gilts were divided into 3 groups while using BCS, only to 2 groups. Gilts from I and II group according to USG were chosen to one group in BCS scale and it was also confirmed by concentration of leptin. Leptin concentration increases in the successive experimental groups with increasing of backfat. The highest concentration was in Gilts group III and this is the only case when the concentration of leptin in gilts was higher than in sows. Unexpectedly the highest concentration of leptin was observed in the group I of sows. In this case (the thinnest sows) it could be explained as a result of using flushing after weaning.
Conclusion: Enhancement of energy level prior to estrus in flush fed sows increases ovulation rate what was confirm in this study. BCS seems to be more reliable in field conditions than instrumental estimation what could be additionally confirmed by presented changes of leptin concentration. Flushing through the short period after weaning didn't increase backfat but change the fat metabolism and reproductive performance.

Disclosure of Interest: None Declared

Keywords: BCS, Leptin, sows

Reproduction

PO-PW1-257

Economical evaluation of altrenogest (Altresyn®) synchronization of gilts in one-week batch management on a large Italian breeding farm

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Introduction: Effective gilt management is important part for breeding efficiency. Oestrus synchronization in gilts by using of altrenogest (Altresyn®, Ceva) is widely used because of proven effectivity for maintain fluent flow of breeding animals in the farm, planning of optimal age of first insemination and life-long productivity. Economic efficiency for the farm is the most important parameter nowadays.
The aim of this study was to compare economic impact of Altresyn® synchronization of oestrus on N° of days-to-scan, cost of the feed consumed and N° of culled gilts because of reproduction reason (not in pig) in comparison with control group of gilts without treatment. Trial was established on large self-gilt producing farm (2000 sows) working in one week batch management system.
Materials and Methods: Animals: 123 cycling gilts from commercial farm self-producing young breeders were included in the study. Altresyn® treatment group TG (n=59) and control group (n= 64) were evaluated simultaneously during the production cycle. Treatment group was individually fed by 20 mg of altrenogest at the same time once daily for 18 days. All the animals received same diet. Days-to-scan were calculated as a time from the second heat detection until the positive pregnancy check. Total sum of those days per group and cost of feed per gilt were calculated.
Results: Six gilts from control group were culled out because of reproduction failure (not in heat), while in altrenogest group 2 animals only.
Return to oestrus parameter was better in Treated Group (1,6%); Control Group showed a 4,6% animals. The difference of 191 days-to-scan in favour for altrenogest group was established, which consequently decreased the cost of feed on level 401 Euro in the treatment group. Total cost per gilt in altrenogest group was lower by 93 € in comparison with group of gilts not synchronized by altrenogest.
Conclusion: We proved in study of two parallel groups of gilts which were introduced in a weekly batch farrowing system the economic benefit of synchronization. The use of altrenogest improved the economical output of the farm by decreasing the number of days to positive pregnancy check on farm, optimizing the consumption of feed in the gilt herd and reducing the number of gilts culled due to reproductive failures. If we take into consideration the average value of a breeding gilt (300 €), the difference and economical benefit is 1200 € in favour to altrenogest treated group. Besides this parameter, the reduction of total costs in the treatment group was calculated on 93 €. Further studies would be needed to assess the impact of the animals on their life performance.

Disclosure of Interest: None Declared

Keywords: Gilts, synchronization

Reproduction

PO-PW1-258

Comparison of the effect of alfaprostol (Gabbrostim®) and cloprostenol farrowing induction on production parameters

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Introduction: Synchronizing parturition has many benefits, such as allowing staff to supervise farrowing, decreasing piglet mortality, minimizing weekend work and cross-fostering management. Alfaprostol (Gabbrostim®) is a synthetic prostaglandin effectively used for this purpose.
Materials and Methods: The study was conducted on a farrow to finish farm in the Northeast part of Italy during spring-summer period. Altogether 250 sows with 3638 piglets were included in the study. Two commercially available prostaglandins were used containing either alfaprostol (Gabbrostim®, Ceva) or cloprostenol. Sows were randomly divided to tree groups: (T1-Control group, n = 53 sows), (T2-Alfaprostol, n = 105 sows) and (T3-Cloprostenol, n = 100 sows). Sows were treated according to the manufacture's reccommendation 24h before the planned time of farrowing. During the analysis, two adjunctive groups were considered: not assisted (farrowing during the night hours) and assisted (farrowing in working hours).
Following parameters were evaluated: Total born piglets, live born piglets and stillborn. In order to evaluate the farrowing, also duration of parturition and N°of uterine explorations in sows needing assistance were recorded. Detailed mortality in the first 5 days of piglets' life was also evaluated with focus on % of crushing and starvation.
Results: The total born and live born piglets were not statistically different among groups, although alfaprostol group delivered in total 0, 8 more piglet per litter in comparison with the cloprostenol group and 1,2 more piglet per litter in the assisted group. Both treatment groups showed statistically lower % of stillborn piglets in comparison with control group. Total mortality of piglets during first 5 days of life was the lowest in the alfaprostol group (8,58%). Assisted farrowing led to decreasing total mortality in both treatment groups and alfaprostol group showed the best performance (7,56 %).
Conclusion: Induction and optimal timing of farrowing and assistance leads to significantly lower stillborn rates in both treatment groups and lower total mortality during the first 5 days of life. Statistically significant difference in total mortality was recorded in the alfaprostol group, mainly driven by lower % of crushed piglets and starvation. Regular use of Alfaprostol can optimize farrowing performance, above all when assistance is guaranteed. Besides the optimal planning of workload in the farrowing house, we consider as a main benefit the positive effect of pre-weaning mortality.

Disclosure of Interest: None Declared

Keywords: None

Poster Abstracts

Reproduction

PO-PW1-248

Faster return to reproduction in primiparous hyper-prolific sows treated with Fertipig®

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Introduction: Meeting breeding targets is the essential element influencing the pig flow in the breeding herd and piglet output. Weaned sows failing to return to oestrus within 7 days after weaning contribute to missed breeding targets and increased non-productive days (NPD) (Patterson 2010). Longer interval between weaning and insemination together with real anoestrus increase the NPDs and contribute to the inability to fill completely the vacant farrowing places. The use of gonadotrophins after weaning may reduce the long weaning–oestrus interval (WOI), prevent anestrus and reduce the number of NPDs. Primiparous sows used to have the tendency to longer WOI and lower ovulation rate due to extensive utilization of body reserves during lactation (Kemp 2004). The aim of the study was to verify if gonadotrophins (Fertipig®, Ceva) can improve the return to reproduction of primiparous sows of current hyper-prolific breeds.
Materials and Methods: A conventional repopulated 900 sow farm practicing 2 weeks batch farrowing system was selected for conducting the trial. In total 177 P1 sows of LW x Landrace were used as non-treated control and 214 P1 sows were randomized according to the backfat thickness and treated with Fertipig® within 24 h after weaning. Heat was detected twice daily within 9 days after weaning. The following litter size was evaluated.
Results: There was no significant difference between the groups as regards to the estrus rate. Fertipig® treated sows had on average 14,2 hours shorter WOI. The difference in WOI between Fertipig® and control was significantly higher in sows of poorer body condition - slim and thin animals (21,29 and 18,69 hours respectively) compared to normal condition sows (10,04 hours).
The % of sows in heat within 5 or 6 days was higher in the Fertipig® group for 11,6% or 23,1% respectively ($p<0,001$).
Sows treated with Fertipig® had on average 0,68 total born and 0,48 live born piglets more than non-treated sows in the following litters.
Conclusion: It was confirmed that in the current high prolific breeds already selected for a short WOI, Fertipig® treatment of primiparous sows can reduce the mean WOI and reduce the number of NPDs. The benefit of lower cost of NPD and 0,48 piglet more calculated using published standards (Ifip 2013) was 27,44€ per sow.

Disclosure of Interest: None Declared

Keywords: primiparous sows, weaning-to-estrus interval

Reproduction

PO-PW1-259

Data confirming confidence in Whole Herd mass vaccination program with Novel PRRS Type 1 modified live vaccine

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Introduction: Vaccination is critical to reduce the severity and frequency of PRRSV-related reproductive problems. Whole Herd mass vaccination is recommended to avoid subpopulations of different immune status. Vaccination during the last trimester of pregnancy is considered most critical because vaccine virus can potentially cross the placenta. Likewise vaccination before breeding could affect litter size. Here a subset of field efficacy data of PRRS genotype 1 MLV vaccine (ReproCyc PRRS® EU) at various stages of gestation was evaluated.
Materials and Methods: Study 1 was conducted on a farm with 660 sows. A total of 283 sows and gilts were included in the study. On study day 0 sows and gilts were vaccinated with 2 ml ReproCyc PRRS® EU. To assess the efficacy of vaccination reproductive performance was recorded from all sows till D119. The percentage of piglets alive per litter at weaning for sows vaccinated in the last trimester of pregnancy (63), compared to the whole group of sows (220) was selected as primary criterion for the evaluation of vaccine efficacy. Secondary criterion was piglets born alive.
Study 2 was conducted on an endemically infected herd with 600 sows. All sows on the farm were vaccinated in a whole herd mass vaccination program with ReproCyc PRRS® EU. The number of piglets alive per litter at weaning for sows vaccinated within 14 days before farrowing (33), or 7 days before insemination (27), compared to the whole group of sows (365) was selected as the primary criterion for evaluation of vaccine efficacy. Secondary criterion was healthy piglets born alive. In addition, the number of piglets born alive was evaluated for naïve pregnant replacement gilts vaccinated at the same day as the sows at various stages of gestation was compared to the whole group of sows.
Results: In study 1, % of piglets born alive was the same for sows vaccinated in the last trimester of pregnancy (92.8) as for the total group of sows (93.2). Also the % of weaned pigs was the same for both groups (90.2% vs 89.1).
In study 2 there was no difference in the number of piglets born alive between sows vaccinated within the last 14 days of pregnancy (12.1), 7 days before insemination (12.1), naïve gilts (10.7) compared to the total group (11.4). Also the number of weaned pigs per litter was the same for sows vaccinated within the last 14 days of pregnancy (10.5) 7 days before insemination (9.9) and gilts (10.0) compared to the total group (9.7).
Conclusion: These studies confirm the confidence in ReproCyc PRRS® EU as a safe vaccine to be used in Whole herd mass vaccination programmes without affecting sows or gilts vaccinated at the most critical time points in their reproductive cycle.

Disclosure of Interest: None Declared

Keywords: mass vaccination, PRRS control, reproduction

Reproduction

PO-PW1-255

The Opportunity Cost of Utilizing Farrowing Room Space as Late-Term Gestation

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Introduction: The wean-to-farrow interval is comprised of two stochastic periods, the wean-to-service interval and gestation length. Variation in the wean-to-farrow interval can be affected by many biological factors as well as by management strategies like induced farrowing, multiple weanings/week and products which facilitate single, fixed-time AI like OvuGel®. The opportunity cost consequence of variation in a sow group's wean-to-farrow interval is the inefficient use of limited farrowing house occupancy resulting in a lower average wean age. Uncertainty regarding an individual's wean-to-farrow interval as well as variation of the interval among animals in the group results in a significant amount of farrowing house occupancy used for late gestation vs. farrowing or lactation. Increasing average wean age beyond threshold levels has been associated with increased wean-to-finish profitability as well as enhanced subsequent sow productivity especially for low parity sows.
Materials and Methods: A stochastic, bio-economic simulation model of the components of the wean-to-farrow interval was created adhering to management rules recommended by modern published standards. The model measures farrowing house occupancy efficiency under different management practices. We utilize up to 440,000 qualified, individual sow records from a large North American data base to estimate distributions by parity (and their correlation) for wean-to-service interval and gestation length. Analogous to non-productive sow days, we estimate for three management scenarios, the percent of available farrowing house days not spent either farrowing or lactating and their impact on average wean age and its standard deviation (STD) as measures of opportunity cost.
Results: Scenario 1: Conventional breeding, single wean/one room; 19.52% farrowing house occupancy as late term gestation. Mean wean age: 18.73 days, STD: 1.74. **Scenario2:** Conventional breeding, two weaning/week; two rooms, 15.23% farrowing house occupancy as late term gestation. Mean wean age: 19.89 days, STD: 1.39. **Scenario3:** OvuGel®, single, fixed-time AI day five post-weaning, induction day 115 for all animals not yet farrowed; 11.79% total farrowing house occupancy days as late term gestation, Mean wean age: 20.82 days, STD: 0.76
Conclusion: **Conclusion:** Use of OvuGel® and single fixed time AI, which facilitated induction at day 115 of gestation, added mean 2.09 days to average wean age while reducing its standard deviation by almost a full day compared to a single wean batch system. The percent of farrowing house occupancy used for late gestation was reduced by 39.6 percent.

Disclosure of Interest: D. DiPietre Conflict with: Consultant to JBS United, L. Mulberry Conflict with: Consultant to JBS United, D. Dau Conflict with: JBS United Animal Health, C. Francisco Conflict with: JBS United Animal Health

Keywords: Opportunity Cost, OvuGel®, Wean age

Reproduction

PO-PW1-241

Using advanced ultrasound technology for managing gilt pool efficiency

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Introduction: Reducing non-productive days has always been an important part of improving gilt and sow productivity. The ability to assess your staff in their effectiveness to accurately identify heats in gilts and validate any replacement gilt hormonal protocols would be invaluable. The use of transcutaneous ultrasound on ovaries and uteri in gilts allows us to observe the puberty status of individual gilts and tailor specific and accurate interventions.
It is not uncommon for producers to have groups of replacement gilts that include both post-pubertal cycling, pre-pubertal non-cycling and gilts of unknown estrus status. A percentage of gilts may also have defects, impossible to acertain by external physical examination, that render them infertile. Having a tool to assess the reproductive soundness and pubertal status of gilts can be invaluable.
Pre-pubertal gilts produce visible small sized follicles on the ovary during development. The pre-pubertal gilt also has a visibly smaller sized uterus. As puberty approaches, medium sized follicles may be seen in some gilts. At time of puberty, there is follicular growth as well as uterine enlargement. Scanning the ovaries and uterus as an indicator for puberty status has proven to be very accurate.
Materials and Methods: A cohort of 24 gilts at 200 days of age were scanned 20 days after arrival to a sow barn to observe their ovaries and uterus using transcutaneous ultrasound technology. After arrival to the sow barn these gilts were housed together in a group pen and received boar exposure on a daily basis. Any signs of heat and date of heat detection was recorded. Observation of puberty status after a 20 day period allowed assessment of the accuracy of what the heat detector recorded and appropriate hormonal intervention to be implemented in a timely manner.
Results: Observation of these 24 gilts revealed that 33% of the group remained pre-pubertal, 63% of the group were post-pubertal and cycling. Staff incorrectly identified the onset of puberty/signs of estrus in 4% of the population as ultrasound scanning identified these individuals as pre-pubertal.
Conclusion: The information provided by transcutaneous ultrasound allowed the producer to make deteriminations regarding the timing of hormonal treatments with minimal negative impact and allowed the heat detector to be confident in observed signs of heat status and areas for improvement.
We need to have effective means by which to provide a constant influx of available gilts most efficiently. Assessment of gilt pool management with the use of a tool such as transcutaneous ultrasound technology on an on-going or intermittent basis would provide a means to achieve such goals.

Disclosure of Interest: None Declared

Keywords: gilt, puberty, ultrasound

Poster Abstracts

Reproduction

PO-PW1-205

The Impact of OvuGel on Reproductive Performance of Weaned Sows

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Introduction: Current artificial insemination (AI) practice is based on multiple AIs with a high number of sperm cells to account for the inability to determine time of ovulation. Synchronizing the estrous cycle of groups of sows is possible through management practices, as well as hormonal intervention. Even with relatively synchronized estrous cycles, wide variation in the moment of ovulation still occurs, precluding single fixed-time AI. A gel for intravaginal application of the GnRH analogue triptorelin (OvuGel) recently became available for induction of ovulation in weaned sows. The objective of this study was to determine the impact of OvuGel on reproductive performance of weaned sows by analyzing data from trials conducted in recent years.
Materials and Methods: Data were from 14 trials conducted in six states in the U.S.A. from 2008 to 2015. A common protocol was followed allowing for the combined analysis. All sows were blocked by parity and lactation length and randomly assigned to OvuGel (OG) or Control treatments. Controls (n=2,924) were AI following normal farm SOP on the day detected in estrus, and 24 hrs later, if still in estrus. All OG sows (n=2,314) were treated 96 hours post-weaning, and AI once 22+/-2 hrs later. Pregnancy was determined by ultrasonography approximately 30 days after AI, and data were collected at farrowing.
Results: Only 90.5% of Controls and 100% of OG sows were AI by day 7 post-weaning with an average of 2.04 and 1 semen doses per sow, respectively (P<0.01). Conception rate (No. pregnant/No. AI) was higher for Controls than for OG sows (91.4% vs. 84.8%, P<0.05). However, when the total number of sows weaned per group was used, conception rate (No. pregnant/No. weaned), was higher for OG sows than for Controls (84.8% vs. 81.9%, P<0.05). Also, farrowing rate (No. farrowed/No. AI) was higher for Controls than for OG sows (89.7% vs. 82.5%; P<0.05). However, when the total number of sows per group was used, farrowing rate (No. farrowed/No. weaned) was higher for OG sows than for Controls (82.5% vs. 80.1%; P<0.05), revealing an increased efficiency of utilization of weaned sows. There was no difference among Controls and OG sows for total number of piglets born per sow (13.2 vs. 13.2; P>0.10) or total number of piglets born alive per sow (12.1 vs. 12.1; P>0.10). OG sows produced 34 more (P<0.04) piglets per 100 weaned sows than controls.
Conclusion: A single fixed-time AI program with OvuGel increases utilization of weaned sows, and consequently farrowing efficiency, without compromising reproductive performance. Enhanced utilization of weaned sows treated with OvuGel resulted in more pigs per weaned sow group.

Disclosure of Interest: None Declared

Keywords: OvuGel, Single fixed time IA, Sows

Reproduction

PO-PW1-211

EFFECT ON SOWS AND LITTERS OF FARROWING INDUCTION BY ALFAPROSTOL VS D-CLOPROSTENOL ADMINISTRATION

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Introduction: Induction of farrowing in sows is usually carried out by administering prostaglandins with the primary objective to increase the synchrony of farrowing. This practice facilitates farrowing supervision, early fostering and 'all in, all out' management of the farrowing house aiming mainly at reducing piglet mortality. The objective of the study was to investigate the efficacy of this practice by using two different commercial prostaglandins: D-cloprostenol and alfaprostol.
Materials and Methods: The study involved 172 sows and their 2084 newborn piglets. Sows were randomly divided into two treatments: Cloprostenol group (n=85 sows) received 0.075 mg (1 ml)/head of D-cloprostenol (Veteglan®-Calier) via IM route; Alfaprostol group (n=87 sows) received 2 mg (1 ml)/head of alfaprostol via IM route. Sows were treated to induce a synchronized parturition 24 h before the expected time of parturition. Sows that farrowed during the diurnal hours were assisted by a trained veterinarian. Sows and litter performances were recorded: farrowing duration; percentage of sows not farrowing in working hours (from 07.00 am to 03.00 pm); number of live-born; total born; stillborn and mummified piglets; number of uterine explorations and piglets mortality during the first 5 days of life (number of dead piglets and cause of death per day). As indicator of perinatal asphyxia, meconium staining on the skin of each piglet was recorded using a three-point scale.
Results: Overall, 70.6% of sows in the Veteglan group and 71.3% of sows in the Alfaprostol group farrowed during working hours, allowing assistance (*P*>0.05). Productive parameters were not influenced by treatment, nor the piglets's mortality. However, Veteglan group showed a lower percentage of crushed piglets (2.15±4.77 vs 5.12±13.78; P<0.001), while a higher percentage of piglets died for starvation (2.25±3.87 vs 1.01±8.65; P=0.029). Furthermore, in the indication of perinatal asphyxia (meconium staining on the skin), piglets belonging to the Veteglan group showed a lower frequency of score higher than 0 (34.6 vs 42.6%; P<0.001). Specifically, piglets with score 2 were 6.25% in the Veteglan group versus 12.07% in the Alfaprostol group (P<0.001).
Conclusion: Both D-Cloprostenol and Alfaprostol were highly efficient in synchronizing sows' farrowing in working hours, allowing an optimal planning of workload on farrowing house and desirable performance in sows and litters. However, Veteglan group showed a lower percentage of crushed piglets and lower frequency of meconium on the skin. Results might suggest a reduced stress on sows and lower asphyxia in piglets during farrowing, even if efforts have to be adopted to improve survival of starved piglets.

Disclosure of Interest: None Declared

Keywords: alfaprostol, D-cloprostenol, farrowing induction

Reproduction

PO-PCO1-017

Induction of ovulation in weaned sows by Porceptal® following different insemination patterns

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Introduction: Reproduction performance plays an important role in the current pig industry. Different synchronization schedules are used. In several studies the GnRH-analog buserelin (Porceptal®) was used. The aim of this study was to assess the achievement using Buserelin following different insemination schemes in German pig herds.
Materials and Methods: The study was conducted in six commercial pig farms in the South of Germany from October 2014 to November 2015. Sows with more than one previous litter were included. The animals were randomly allocated into three groups. The sows of group A (n=240) received 2.5 ml Porceptal® (10 µg of Buserelin) at 83-89h after weaning and a single fixed time artificial insemination (AI) followed 30-33h later. The sows in group B (n=60) were given Porceptal® 83-89h after weaning and were inseminated three times 24-26h, 36-38h and 48-50h after the injection. Group C (n=150) represented the control group. These sows received PMSG 24h after weaning and an application of hCG 72h after PMSG injection. The animals were inseminated 16h, 24-26h and 40h after hCG injection. The level of significance was set to $p < 0.025$ due to bonferroni correction. Both Porceptal® groups were compared with the control group by comparing the number of sows tested pregnant by ultrasound.
Results: The sows showed a distinct standing heat. Using a single fixed time AI resulted in an increase in the return to estrus index. Group A with a single fixed time AI showed a significant difference in comparison to group C ($p < 0.001$). Comparable results could be seen between the control animals (group C) and the sows inseminated three times (group B) ($p= 0.301$). The percentage of sows that returned to estrus was 20.0% in group A, 8.3% in group B and 4.0% in group C.
Conclusion: Buserelin as Porceptal® triggers heat and subsequently successful ovulation. In this study a reliable insemination wasn´t possible ($p < 0.001$) with a single fixed time AI. The study confirmed that the given application schedule is reliable to successfully inseminate if a fixed time AI is used at three times. The results of the Porceptal® group B are similar to those of group C.

Disclosure of Interest: None Declared

Keywords: buserelin, insemination, synchronisation

Reproduction

PO-PW1-210

Induction of ovulation by using GnRH agonist in weaned sows in Thailand

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Introduction: For artificial insemination (AI) in general commercial swine herds, the sows are inseminated 2 to 3 times during standing estrus. The reason is due to the fact that the ovulation time is unpredictable. The variation of ovulation time is caused by the variation of estrus to LH surge interval. GnRH is a hormone induced FSH and LH secretion and stimulate follicular growth and ovulation. The objective of the present study was to determine the ovulation time after GnRH agonist administration in sows under tropical climates.
Materials and Methods: In total, 43 Landrace x Yorkshire (LY) crossbred weaned sows in a commercial swine herd in the southern part of Thailand were included. The sows were kept in evaporative cooling system. Standing estrus was determined twice a day, at 8.00 am and 16.00 pm, by using back pressure test in front of a mature boar. The sows were classified into 2 groups: control (n=20) and treatment (n=23). The treated sows were intramuscularly injected with 10 µg (2.5 mL) of buserelin (Receptal®, MSD, USA) at 72 h after weaning. Ovulation was examined in each sows every 8 h after the onset of estrus by using transrectal ultrasonography (HS-2000, Honda, Japan) at 5.0 MHz. Wean-to-estrus interval (WOI) and estrus-to-ovulation interval (EOI) were compared by using Student's *t* test.
Results: The reproductive parameters of the sow in both groups including parity number (2.6 and 2.9), backfat thickness (16.7 and 17.5 mm), lactation length (20.5 and 20.9 d) and WOI (3.3 and 3.6 d) were not significantly difference between control and treatment groups, resp. ($P>0.05$). The treated sows exhibited standing estrus at 16.7±4.1 h after GnRH treatment. The treated sows had a shorter EOI than the controlled sows (35.4 ± 15.1 and 65.4 ± 19.2 h to 38.4 ± 13.1 and 64.9 ± 19.8 h). In the treatment group, ovulation took place at 52.5 ± 13.6 h after GnRH treatment. Cystic ovaries was found in both the control (5.0%) and the treatment (8.7%) groups. These data indicate that ovulation time can be efficiently controlled by using 10 µg of buserelin in tropical sows.
Conclusion: Under tropical climates, normal sows ovulated at 64.9 h after the onset of estrus. The treatment of 10 µg of buserelin at 72 h after weaning resulted in standing estrus at 14.1 h after the hormone injection and ovulation took place at 38.4 h after the onset of estrus.

Disclosure of Interest: P. Pearodwong Conflict with: Royal Golden Jubilee (RGJ) Ph.D. Program, Thailand Research Fund, P. Tangjarernbumrungsuk: None Declared, P. Limpiwattakee: None Declared, S. Manop: None Declared, O. Mora: None Declared, P. Tummaruk Conflict with: Royal Golden Jubilee (RGJ) Ph.D. Program, Thailand Research Fund, C. Tretipskul: None Declared

Keywords: GnRH, Ovulation induction, Sow

Poster Abstracts

Reproduction

PO-PW1-246

Total non-productive days and its categories are improving in Colombian farms in the period 2011-2014

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Introduction: The analysis of Non-Productive days (NPD), including its breakdown by types is a very useful tool to understand reproduction problems, but it is not usually performed probably because of its complexity and lack of availability in most swine software packages. This is even more unusual in small familiar farms that lack of opportunity of having routinely professional support. The objective of this abstract is to characterize the distribution and trend of the different types of NPD at Colombian farms controlled and supported by ASOPORCICULTORES -Fondo Nacional de la Porcicultura (FNP).
Materials and Methods: A total of 42 Colombian farms, including 14.100 productive sows, were grouped in a single database including data from October 2011 to September 2014 to calculate the total number of NPD and its distribution in six key intervals: wean-first service (WFS), first service-effective service (FSES) of multiparous, first service-removal of multiparous (FSR), wean-removal (WR), FSES of gilts and FSR of gilts.
The analysis was split in three periods of 12 months. Data were analyzed using PigCHAMP software and by GLM procedure of SAS.
Results: The average number of NPD per sow and year were 68, with a linear trend to decrease over the three studied periods: 71.0, 70.1 and 63.4, respectively ($p<0.05$). From these 68 NPD, 37.6 are due to gilts still non-productive (intervals arrival-first service 35.7 days and arrival-removal 1.9), and 30.4 to sows. The averages of NPD per sow and year for every interval are: 11.8 WFS, 6.4 FSEF of multiparous, 5.7 FSR of multiparous, 3.2 WR, 2.1 FSES of gilts and 1.3 FSR of gilts.
Overtime, most of the key intervals are improving, in particular those related to 1st service- effective service and 1st service to removal, both in gilts and sows ($P<0.05$), what can be explained by a better efficiency in reproduction management and the efficiency in the time on farm before culling.
It must be remarked that wean to first service is contributing with highest NPD per sow and year since almost all the sows have 1-2 wean to first service intervals during a year. Thus, a low decrease of wean to first service interval will probably suppose a higher reduction of the overall average NPD/sow/year of a farm than higher decreases in other intervals.
Conclusion: NPD are improving in the period 2011-2014 in small-familiar farms in Colombia belonging to the program coordinated by ASOPORCICULTORES-FNP, mainly due to a better efficiency in gestation and a lower time before culling in farms. The use of this analysis in a routine is a good tool to improve the competitiveness of farms, including local industry based on small-familiar farms.

Disclosure of Interest: None Declared

Keywords: Non-productive days, sows

Reproduction

PO-PW1-204

Total born and alive born piglets in the first farrowing are good indicators of sows' lifetime performance in large Brazilian farms

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Introduction: Total born piglets (TB) in the first farrowing have been recently described as a reliable indicator of sows' lifetime performance in southern EU farms. The objective of this abstract is to analyze this effect in large Brazilian swine arms including other key productive indicators such as born alive piglets (BA) and still birth (SB).
Materials and Methods: A total of 4 Brazilian farms, including 14.727 productive sows, were used for the study and included in a single database, including data from the first farrowings occurred in the period 2005-2010 and the sows' later lifetime performance, for a total of 183.337 farrowings. Linear effect between data in the first farrowing and lifetime performance of sows were assessed by REG procedure of SAS (v 9.0).
Results: TB piglets at first farrowing has a clear influence on subsequent TB, with a significant linear effect ($p<0.01$) and a R^2 of 0.342. The effect is similar for BA piglets, showing a linear effect ($p<0.01$) with R^2 of 0.342. In the case of SB piglets the pattern is similar, having a linear effect ($p<0.01$) and R^2 of 0.282 but, it must be remarked that if sows that only had one parity (normally because of reproductive problems) are excluded from the analysis, the effect is less clear, and the R^2 decreases to 0.173. This means that the effect for SB is supported by a number of problematic sows that influence largely the overall results. Without first farrowing problematic sows, correlation is much weaker.
Conclusion: TB and BA piglets in first farrowing are a good predictor of sows' lifetime performance in large Brazilian swine farms. SB is not a strong reliable indicator since it is influenced by sows discarded having only one parity. Based on this, focusing on getting a good first farrowing and adjust culling policy to TB and BA (excluding SB) could be a good framework to improve reproductive performance.

Disclosure of Interest: None Declared

Keywords: None

Reproduction

PO-PW1-252

Control of parturition in sows by using PGF2 in combination with carbetocin

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Introduction: Intensive farrowing management as well as care of newborn piglets lead to an increase in the number of piglets born alive per litter. Control of parturition by using prostaglandin F_{2a} (PGF_{2a}) is commonly used in swine commercial herds worldwide. The injection of oxytocin at 20-24 h after $PGF_{2\alpha}$ has been used to prompt uterine contraction and improve the predictability of the farrowing times. However, the adverse effect is also observed, e.g, an increase number of stillborn and piglets with umbilical cord ruptured. Carbetocin is an oxytocin-like synthetic substance which action on the same receptor on myometrium. In human, carbetocin is recommended to use to prevent postpartum hemorrhage. The use of carbetocin in pig is rather limited. The aim of the present study was to investigate the induction of parturition protocol by using $PGF_{2\alpha}$ in combination with carbetocin in pigs.
Materials and Methods: The experiment was performed in a commercial swine herds in the western region of Thailand. In total, 187 Landrace x Yorkshire crossbred sows were included. The animals were randomly assigned into one of three groups: control (natural farrowing), PGF_{2a} alone, and $PGF_{2\alpha}$ in combination carbetocin. The PGF_{2a} was administrated intramuscularly by using a synthetic analog of PGF_{2a} (175 µg Cloprostenol, MSD, USA) at 8:00 AM on day 114 of gestation. In PGF_{2a} + carbetocin group, 0.6 µg/kg of carbetocin (0.05 mg/ml Decomoton®, Barcelona, Spain) was administered intramuscularly at 24 h after PGF_{2a}. Farrowing process was monitored 24 h. The sows that farrow during the period from 7 AM to 5 PM were defined as farrowing during the working hours. The onset of farrowing, the farrowing duration and the birth interval between each individual piglets was recorded. The data were analyzed by using ANOVA and Chi-square test.
Results: The percentages of sows farrowed during the working hours in natural farrowing, induced farrowing using $PGF_{2\alpha}$ alone and $PGF_{2\alpha}$ + carbetocin were 51.3%, 70.0% and 98.1%, respectively. The average of birth interval was 20.7 min. The birth interval did not differ significantly between natural farrowing (17.1 min) and induced farrowing using $PGF_{2\alpha}$ (20.7 min) or $PGF_{2\alpha}$ + carbetocin (19.3 min). Likewise, the farrowing duration did not differ significantly between between natural farrowing (4.8 h) and induced farrowing using $PGF_{2\alpha}$ (4.9 h) and $PGF_{2\alpha}$ + carbetocin (4.3 h).
Conclusion: Farrowing induction at 114 days of gestation by using PGF_{2a} in combination with carbetocin to control the time of farrowing in sows is more effective than using PGF_{2a} alone.

Disclosure of Interest: None Declared

Keywords: carbetocin, induced parturition, pig

Reproduction

PO-PW1-222

Effect of three seminal plasma proteins on pig semen freezability

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Introduction: The results of fertility with the application of frozen-thawed boar semen are not consistent due to between-boar variation in freezability. The objective of this work was to determine the effect of three seminal plasma proteins on freezability of boar semen: Nieman-pick disease type C2 (NPC2), cholesterol transfer in cell membrane; heat shock protein 90 alpha A1 (HSP90AA1) and Lipocalin-Type prostaglandin D synthase (PGDS-L) involves in capacitation and acrosome reaction.
Materials and Methods: Semen of six boars was frozen three times in a row and evaluated after thawing to ascertain freezability through measurement of the percentage of sperm functionally competent (SFC). One boar was selected with the highest freezability (BHF) and one with the lowest freezability (BLF) for semen collection and immediate separation of sperm from seminal plasma (SP). Four combinations of sperm and SP were incubated during three hours lowering the temperature to 17ºC before freezing, and evaluated post-thawing for morphology, membrane structural integrity, total and progressive motility, acrosome integrity, functional integrity of membrane and SFC. Protein identification was performed through SDS-PAGE and Western blot to quantify at zero and three hours of incubation time, based in volume and density band. 2x2x2 factorial arrangement in completely randomized design was performed. ANOVA and correlation analysis were performed.
Results: two isoforms of the protein NPC2 (16 and 19 KDa) were detected. The interaction sperm x incubation time had an effect on HSP90AA1 ($P < 0.01$), and PGDSL ($P < 0.05$) levels, and did not ($P > 0.05$) on the levels of isoforms NPC2; SP x incubation time interaction only affected ($P < 0.01$) NPC2 of 19 KDa levels; SP factor did not affect the PGDS-L and HSP90AA1 levels, and the sperm factor did not affect the NPC2 of 19KDa ($P > 0.05$). The NPC2 of 16 KDa was affected ($P < 0.01$) only by incubation time, with a reduction after three hours. NPC2 of 19 KDa concentration was highest in SP of BHF combinations ($P < 0.01$) and it concentration decreased at the end incubation time. After of the incubation, the HSP90AA1 concentration increased ($P < 0.01$) in greater amounts in sperm of BLF combinations; and PGDSL concentration declined most in these same combinations. Only there was a negative correlation between the levels of HSP90AA1 and progressive motility (-0.6, $P > 0.05$).
Conclusion: The type of sperm or seminal plasma, whether high or low freezability, affected the concentration of the three seminal plasma proteins, indicating an association of these with freezability of boar semen.

Disclosure of Interest: None Declared

Keywords: pig, semen preservation, seminal plasma

Poster Abstracts

Reproduction

PO-PW1-247

Serum estradiol-17β concentration after estrus induction using PG600® in gilts

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Introduction: PG600® is an exogenous gonadotropin hormone combination of Pregnant Mare Serum Gonadotropin (PMSG) and Human Chorionic Gonadotropin (hCG). This combination hormone is commonly used for estrus induction in prepubertal gilts and weaned sows. Studies found that 50-70% of gilts treated with PG600® exhibit standing estrus within 4-7 days after administration. However, the variation of gilts response to PG600® is still not completely understood. The objective of the present study was to determine the serum estradiol-17β concentration in gilts after induction of estrus by using PG600®.
Materials and Methods: Thirty-five Landrace and Yorkshire crossbred gilts were included in the experiment. Gilts were weighted, measured for backfat thickness. Trans-abdominal ultrasonography was performed to determine the reproductive status. The gilts were administrated with PG600® intramuscularly. Heat detection was performed using fence-line boar exposure with back pressure test for 7 days consecutively. On Days 0 and 3, blood samples were collected by jugular venipuncture. Serum was transferred into polypropylene microtubes and storage at –20°C until estrogen analysis. Serum was purified and concentrated using diethyl ether extraction. Serum estradiol-17β was determined using a commercial Enzyme Immunoassay kit (DetectX® serum 17β-estradiol EIA kit, Arbor Assays, USA) in duplicated samples. Data were analyzed using SAS. Descriptive statistics were calculated. The concentration of estradiol-17β at Days 0 and 3 were compared using paired *t* test. $P<0.05$ were regarded to be statistically significant.
Results: On average, the age, body weight, backfat thickness and average daily gain of the gilts were 189.2 day, 101.3 kg, 8.2 mm and 537.9 g/day, respectively. Of all gilts, 16 gilts (46%) exhibited standing estrus within 7 days after PG600® administration. The difference of serum estradiol-17β concentration among groups was observed. Average serum estradiol-17β on day 0 and day 3 were 9.8±0.9 and 26.0±3.5 pg/mL, respectively. Compared between Days 0 and 3, the serum estradiol-17β concentration was significantly increased (+21.4 pg/mL, $P<0.001$) in the gilts that exhibit standing estrus, but was not increased (+10.7 pg/mL, $P=0.06$) in the gilts that did not exhibit standing estrus.
Conclusion: The present study demonstrated the variation of estrus expression in gilts after PG600® treatment. For the gilts that did not exhibit standing estrus, the level of serum estradiol-17β at 3 days after treatment might be too low to initiate the estrus behavior. Thus, the mechanism of estrogen synthesis in the ovary should be further investigated.

Disclosure of Interest: None Declared

Keywords: estradiol, estrus expression, PG600

Reproduction

PO-PW1-240

Sow group housing during early gestation; room for improvement

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Introduction: In 1998 the Dutch government issued that from 2013 sows must be housed in social groups within 5 days after insemination, in addition to EC directive 2008/120. In 2014, the government issued a project to aid farmers, which had reported, on request, negative effects of group housing in the first 4 weeks on welfare and reproduction (n=347), to overcome these effects. Focus of the project was on the interventions to chronic physical stress, as this is an important risk factor for negative outcomes of reproduction.

Materials and Methods: The project is based on 3 pillars:
1) A multi-disciplinary project team of 8 independent swine professionals (3 vets, 3 welfare scientists, a feed and a swine management consultant), was asked to visit 222 farms that requested help from experts between mid-2015 and mid-2016. Firstly, an anamnesis was taken by phone to define the exact problem field ((re)production, aggression, lameness and sow mortality). Subsequently, 2 experts visited the farm and assessed farm and animal management and evaluated risk factors e.g.: body condition in the farrowing unit, hunger, feeding system, behaviour around feeding times, socialisation of gilts, seasonal effects, aggression, space allocation and grouping management, etc. The farmer was encouraged to invite their farm advisors to attend during the expert farm visit. A tailor made advice was given and the implementation of the advice was evaluated by phone 3 months later.
2) 4 regional meetings to disseminate knowledge on sow group housing for all swine farmers and farm advisors.
3) A website with public access to practical information:
http://www.wageningenur.nl/nl/show/Groepshuisvesting-zeugen-in-de-vroege-dracht.htm

Results: As of jan-2016 90/222 farms were visited. In 92% of visited farms, at least one or more risk factors could be identified. Advice varied from minor changes in farm management to significant changes in design of housing or feeding. Farms with through or floor feeding of sows are overrepresented in the latter group.

Conclusion: Literature has shown that group housing of sows in early gestation is feasible, but one should take into account sow's needs to reduce chronic physical stress. Mid-term evaluation of the project shows that the design of the project enables to identify plausible causes of the problems in a majority of the farms as well as that some regular farm advisors needed to update their knowledge on individual sow's needs in order to formulate effective advice on this matter.

This project is financed by the Dutch Ministry of Economic Affairs

Disclosure of Interest: None Declared

Keywords: Early gestation, Group housing, Sow Performance

Reproduction

PO-PW1-242

Effects of omega-3 supplementation on boar sperm quality

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Introduction: As artificial insemination is largely used in swine, strategies to optimize sperm quality are of interest, since boar sperm is sensitive to damages due to cold shock and oxidative stress (2). Supplementation of diets with polyunsaturated fatty acids of the omega-3 series appear to benefit female reproductive performance, but their effects on boar sperm quality are not yet clarified. This study evaluated the effects of omega-3 supplementation in diets on boar sperm quality.
Materials and Methods: Six crossbred adult boars with known fertility were used. The boars were split in two groups (n = 3 each): a control that were fed 2.8 kg/d of a diet including 16.0% CP and 3,200 kcal ME/kg; and a group that received the same diet supplemented with 150 g/kg of seaweed meal derived from the microalgae *Schizochytium sp* (4), containing 120 g of docosahexaenoic acid (DHA) per kg, totaling 18% DHA, during 75 d.
Sixteen semen samples were collected per boar, from 7 d after the beginning of the supplementation, until 60 d after its conclusion. Semen was diluted in BTS (3), with 3 x 10^9 spermatozoa in 100 mL. Sperm motion parameters were determined by a computer assisted semen analyses system (SpermVision®, Minitube), using 6 automated randomized fields and compared between groups using ANOVA with repeated measures. Responses were transformed to arcsine or log when lack of normality was detected.
Results: For omega-3 supplemented boars, distance average path (in both a curved and in a straight line), velocity average path (in both a curved and in a straight line), amplitude of lateral head displacement and beat cross frequency were lower than for the control group ($P < 0.05$). No differences ($P > 0.05$) were observed for total and progressive motility, straightness, linearity, wobble and beat cross frequency.
Conclusion: Compared to the control, spermatozoa produced by omega-3 supplemented boars presented similar motility, but were slower and traveled lower distances. Generally, studies dealing with omega-3 effects on boar sperm quality revealed contradictory results. Beneficial effects were reported (5, 6), as well as potential toxicity (6) and lack of effects on sperm quality (1). Further studies in this filed are still required.

Disclosure of Interest: None Declared

Keywords: boar, omega-3, semen

Reproduction

PO-PW1-231

Ultrasonography evaluation of ovary and uterus avoids the inclusion of not pubertal gilts in a program of oestrus synchronization with altrenogest.

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Introduction: The administration of Altrenogest (ALT) is an effective system to synchronize oestrus gilts but it has to be utilized only in pubertal animals. Usually, it is difficult to precisely know the reproductive status of gilts and some animals may be treated with ALT even if they are not pubertal. The aim of this study has been to differentiate pubertal from not-pubertal gilts by ultrasound (US) evaluation of ovary and uterus before a program of oestrus synchronization with ALT.
Materials and Methods: In this study 210 crossbred gilts of 8 months of age were divided in two group: 108 gilts (ALT+US group) were examined by US (Honda HS-1600V, convex probe: 9.0/7.5/5.0 MHz) and diagnosed as no-pubertal or pubertal. Gilts were considered as ‘‘pubertal’’ in the presence of: i) follicles >6 mm or/and of CLs; ii) a uterus diameter more then 1.2 cm2 that extends into the caudal abdomen iii) clearly shaped uterine loops. Gilts were considered no-pubertal: i) in absence of CLs and with follicles < 5 mm; ii) with a uterine diameter less than 1.2 cm2 that occupies only a minor space and that is located very close and cranial to the urinary bladder, iii) with the uterine loops that are closely attached to each other, appears to be partially over-lapped and are not visualized as clearly separated cross-sections. Thereafter, only pubertal gits were treated with 15 mg ALT (Regumate®) for 18 days. A second group of 102 gilts (ALT-NO-US group) were not evaluated with ultrasound but directly treated with ALT.
Results: In ALT+US group 18/108 (16%) gilts were considered as not-pubertal and excluded from the treatment with ALT. The remains 90 gilts were synchronized and 82 (91%) went in oestrus and 72 (94%) become pregnant. In ALT-NO-US group, 89/102 (87%) gilts after ALT treatment were in oestrus and 77 (86%) become pregnant.
Conclusion: The results of this study confirm previous data from literature that ultrasonography of ovary and uterus is an appropriate method to distinguish between not-pubertal and pubertal gilts and avoids the inclusion of not pubertal gilts in a program of oestrus synchronization with altrenogest.

Disclosure of Interest: None Declared

Keywords: ultrasonography, gilts, puberty, synchronization.

Poster Abstracts

Reproduction

PO-PW1-243

Presence of leptin and its receptor in preantral follicles of prepubertal gilts supplemented with omega-3

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Introduction: Leptin and its long form receptor (ObRb) are present in the hypothalamus of cyclic females and prepubertal gilts (3), signaling their nutritional status to the reproductive axis. Supplementation of diets with omega-3 polyunsaturated fatty acids (PUFA), especially the eicosapentaenoic (EPA) and the docosahexaenoic (DHA), may affect leptin plasma concentration (2). This study evaluated the effect of omega-3 supplementation on the leptin and ObRb immunolabeling in oocytes of prepubertal gilts.
Materials and Methods: Prepubertal finishing gilts from a commercial farm were fed 2.5 kg/d of a diet with 14.7% CP and 3,205 kcal/kg ME and received orally either soybean (n = 13) or fish oil (n = 12), with a disposable syringe (9 ml of each oil) during 45 d. The soybean oil had 8.3 Kcal (35.0 Kj) in 1.0 g, with 0.9 g of total fat (0.55 g of unspecified PUFA, but no omega-3). The fish oil had 9.5 Kcal (40.0 Kj) in 1.0 g, with 0.9 g of total PUFA (0.35 g DHA and 0.5 g EPA).
Blood samples were collected at D0 and D45. Serum leptin levels were determined by ELISA. For immunohistochemistry, 3 µm slices of ovarian tissue were collected at slaughter from four gilts per group (4). Images were captured with a digital camera attached to a light microscope, at 40X, for oocytes included in primordial/primary (OIPF) and secondary (OISF) follicles, and at 10X, for oocytes in tertiary follicles (OITF). The means of mode immunolabelling values for leptin and ObRb and of leptin serum levels were compared between groups using repeated measures ANOVA.
Results: Leptin serum levels did not differ between groups ($P > 0.05$). For supplemented gilts, leptin immunolabeling in OIPF and immunolabeling for both leptin and ObRb in OISF was more intense than for controls ($P < 0.05$). However, in OITF, immunolabeling for leptin and ObRb was similar between groups ($P > 0.05$).
Conclusion: The intense immunolabeling for leptin and ObRb in OIPF and OISF suggests that omega-3 may play a role on oocyte competence through leptin metabolism in the ovaries. Though, in prepubertal gilts, oocytes are less competent than in pubertal gilts (1) and leptin immunolabeling is less intense than in similar oocytes of sows (4). As leptin serum levels were unaltered across groups, the role of omega-3 on the ovarian function of prepubertal gilts still deserves further research.

Disclosure of Interest: None Declared

Keywords: Leptin, omega-3, ovaries

Reproduction

PO-PW1-218

Fixed-Time-Insemination after synchronization of ovulation with Porceptal®: Comparing different time schedules in a system with morning weaning

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Introduction: Fixed time insemination (FTI) is based on a treatment with GnRH-analogue Buserelin (Porceptal®) to induce ovulation in gilts and sows, followed by a single artificial insemination (AI) at defined time intervals. The current schedule is, however, not labor- friendly in a system with morning (AM) weaning due to working hour constraints. The aim of this study was to test different time intervals between weaning, GnRH application and AI to make FTI more labor-convenient.
Materials and Methods: Study was conducted in a German 1,600 sow farm with Danish Genetic, weekly farrowing and an average 25 day lactation length. A total of 134 sows (parity 2-5, average: 4.4) were included and randomly allocated to one of three treatment (EG1-3; n=69) and control groups (CG1-3; n=65). Weaning was done at 7 AM (EG1: n=17; CG1: n=16), 8 AM (EG2: n=25; CG2: n=25) or 10 AM (EG3: n=27; CG3: n=24). EG1-3 received 10 µg Buserelin (2.5 ml Porceptal®) i.m. at three different time intervals post weaning: 96 (EG1), 94 (EG2) and 92 h (EG3) and were inseminated once either 24 (EG1) or 30-33 h (EG2&3) after GnRH injection. CG1-3 were not treated and inseminated according to standing heat on an AM/PM schedule (average 3.1 AI/sow). Treatments and AI occurred within normal working hours (7 AM-4 PM). Diluted semen of 13 different boars was used and randomly allocated to EG and CG with only one boar per sow. Backfat (BF) was measured prior to farrowing and post weaning. Ultrasound was applied to monitor follicles at GnRH-injection and ovulation at four different time points. Pregnancy and farrowing rates as well as litter sizes were recorded.
Results: Mean BF prior to farrowing (13.6 vs. 13.5 mm) and at weaning (10.0 vs. 10.4 mm) was similar for EG and CG. Follicle sizes did not differ at GnRH injection (5.5mm) and 30 h later (5.9 mm). More EG had ovulated within 48 h after GnRH injection than CG (98.6 vs. 81.0%). At 72h post injection, all ES and 92.1% of CG had ovulated. In EG 1-3, 76.5%, 92.0% and 96.3% were checked pregnant, of which 76.5%, 92.0% and 81.5% farrowed (three EG3 pregnant sows failed to farrow due to death or abortion). In CG 1-3, 93.8%, 100% and 100% were pregnant and 87.5%, 92.0% and 91.5% farrowed. Litter sizes were similar across groups. Mean total and liveborn piglets for EG were 20.1 and 17.4, and for CG 19.7 and 16.5.
Conclusion: FTI is feasible in a system with Danish genetic, an average 25 day lactation length and a batch farrowing system with morning weaning, and ensures good fertility results. In this study, the 92 h time interval between weaning and GnRH and AI 30–33 h later proved to be the optimum time schedule based on superior pregnancy rates.

Disclosure of Interest: None Declared

Keywords: Sow; Porceptal; Fixed-time-Insemination

Reproduction

PO-PW1-215

Fixed-Time-Insemination after synchronization of ovulation with Porceptal®: Fertility results of a modified time schedule in a system with AM weaning

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Introduction: Fixed time insemination (FTI) is based on a treatment with the GnRH-analogue Buserelin (Porceptal®) in order to induce ovulation in gilts and sows, followed by a single FTI both at defined time intervals. Since the current time schedule is not very labor-convenient in a system with AM weaning due to working hour constraints, a 92 h interval between weaning and GnRH injection was evaluated and the outcome suggested superior fertility results. This study was aimed to test this time schedule on a larger number of sows in a field experiment.
Materials and Methods: The study was conducted in a German 1,600 sow farm with Danish Genetic, weekly farrowing and an average lactation length of 25 days. A total of 178 sows (parity 2-5, average: 4.3) from 6 batches were weaned at 10 AM on Thursday and randomly allocated to experimental (EG; n = 94) and control (CG; n = 84) groups. EG received 10 µg Buserelin (2.5 ml Porceptal®) i.m. on Monday at 6 AM 92 post-weaning while CG remained untreated. EG were artificially inseminated (AI) once on Tuesday between 12 AM and 3 PM (30-33 h post GnRH), while CG were inseminated according to standing heat on an AM/PM schedule (average: 3.4 AI/sow). Semen from 11 different boars was used for AI and randomly allocated to EG and CG, with only one boar per sow. Backfat (BF) was measured prior to farrowing and at weaning. Pregnancy and farrowing rates as well as litter sizes were recorded, and piglets were weighed at birth.
Results: Mean BF prior to farrowing was15.4 mm (14.4 vs.16.3 mm for EG and CG) and at weaning 12.6 mm (11.6 vs. 13.7 mm for EG and CG). Mean BF loss in lactation was 2.9 mm in EG and 2.6 mm in CG. Mean pregnancy rates for EG and CG were 92.6% and 98.8% (range: 84.2-100% for EG and 94.1-100% for CG). Mean farrowing rates for EG and CG were 89.4% and 94.4% (range: 76.9-100% for EG and 85.7-100% for CG). Mean total and liveborn piglets for EG were 18.9 (range: 2-28) and 16.2 (range: 2-24), respectively, and for CG 20.1 (range: 7-29) and 17.1 (range: 0-27). Mean piglet weight at birth was 1.32kg (range: 0.81-2,19kg) for EG and 1.25kg (range: 0.81-1,67kg) for CG.
Conclusion: The results support that**,** in a system with Danish genetic, an average 25 day lactation length and a batch farrowing system with morning weaning, a 92 h interval between weaning and GnRH injection and a single AI 30-33 h later give fertility results almost as good as in sows bred according to the standard farm schedule. Although litter sizes were slightly lower with single FTI, birth weights were moderately increased.

Disclosure of Interest: None Declared

Keywords: None

Reproduction

PO-PW1-235

Bacterial contamination of boar semen, antibiotics use and Dicol® efficacy

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Introduction: Bacterial contamination in extended porcine semen occurs frequently in artificial insemination centers. The aim of this study is to check the main bacterial species isolated from seminal doses, to assess the activity of antibiotics, to check the appropriate combination of antibiotics to control bacteria and to demonstrate the efficacy of Dicol®.
Materials and Methods: The study was carried out with commercial extended semen. Bacterial cultures, isolation and identification were performed. Antibiograms for all isolated bacteria were carried out. Semen samples collected into extender free of antibiotics were infected with 10^6 ufc/ml of two strains: *Serratia marcescens (gram-negative), and Micrococcus spp. (gram-positive).* Infected semen samples were diluted to reach 30 millions sperm/ml and incubated with 4 increased concentrations of 10 antibiotics (A1 to A10). All samples were incubated at room temperature and cultivated for bacteria content assessment after 50 min and 24 hours of treatment. Finally, five ejaculates from five different boars were collected and divided into 3 groups which were initially diluted (1:1) with Dicol, Vitasem or Duragen extender. Subsequently, all groups were divided into 10 aliquots, and infected with 10^6 cfu/ml of final concentration from 10 pure isolated multi-resistant strains of bacteria. After 25 or 50 min, final dilution was carried out with Duragen, Vitasem or antibiotics free extender to reach a $3x10^7$ spermatozoa/ml. All samples were stored at 16ºC and bacterial load determined at 24 hours.
Results: Semen culture showed that 26.24% of examined samples were infected. 113 species of bacteria were isolated and cloned. The most commonly found in extended semen samples were: *Proteus vulgaris* (3.5%), *Serratia marcescens* (6.19%), *Serratia liquefaciens* (12.39%), *Staphylococcu spp* (9.4%), *Cedea* (4.4%), *Micrococcus spp* (5.3%), *Morganella mornanii* (4.4%). The results of the controlled infections showed a significant reduction in both bacteria content after 50min of incubation with some antibiotic concentrations. Interestingly, *Serratia marcescens* and *Micrococcus spp* content was <1ufc/ml after 50 min of treatment with 25x MICs concentration of A1, A3 and A10, which allowed to antibiotic combination and Dicol design for bacteriospermia treatment. The results of Dicol efficiency showed almost no growth (<1cfu/ml) of bacteria in samples of Dicol group even in the case of final dilution with antibiotics-free extender.
Conclusion: The analyses of semen contamination, antibiograms, controlled infection by *Serratia marcescens,* and *Micrococcus spp* and the results of antibiotics concentrations effects permitted to develop an effective combination of antibiotics and Dicol design.

Disclosure of Interest: None Declared

Keywords: None

Poster Abstracts

Reproduction

PO-PW1-234

EFFECT OF SILVER PARTICLES IN BOAR SPERM

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Introduction: Silver nanoparticles (AgNPs) are currently being very widely used in industry, mainly because of their anti-bacterial properties, with applications in many areas. Silver nanoparticles (AgNPs) are among the most popular nanomaterials used in biological science. Therefore, exposition to AgNPs may have harmful effects. The mechanism by which AgNPs can induce cytotoxicity is thought to be by apoptosis induction.
The purpose of this study was to investigate the effect of AgNPs in contact with boar spermatozoa and the effect in the sperm quality.

Materials and Methods: Five ejaculates were extended with Duragen® and treated with different AgNPs (Silver proteinate, electrolytic silver, Argemag-KA-HF-5.9, Argemag-5, Argemag-6, Argemag-7, Argemag-KA-5.9, AgNPs < 100nm and Silver oxidum), storage at 16 ºC for a week. A sample without AgNPs was used as control. To evaluate sperm quality, several parameters including viability, mitochondrial potential, acrosome integrity and early apoptosis by flow citometry and motility by CASA system were analyzed at 1, 4 and 7 days.
Results: Samples treated with different AgNPs showed similar motility (87% vs. 92%), than the control samples, but less viability (60% vs. 80%), mitochondrial potential (58% vs. 81%), high percentage of reacted acrosome (29% vs. 8%) and high percentage of early apoptosis rate (50% vs. 10%) compared with control sample at 4 and 7 days. There are no differences between treated and control sample at 1 day of storage except for Argemag-6, which was the most harmful silver nanoparticle.
Conclusion: AgNPs damage sperm membrane, affect to the mitochondrial activity, increase acrosome reaction and induce early apoptosis. AgNPs compromise highly sperm quality and activated the apoptotic process.

Disclosure of Interest: None Declared

Keywords: silver nanoparticles, reproductive toxicity, sperm

Reproduction

PO-PW1-249

STUDY OF SOWS AND BOARS GENITALIA ELIMINATED FROM FARMS AND ARTIFIFICIAL INSEMINATED CENTERS

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Introduction: Post-mortem collection of the sow and boar genitalia in the slaughterhouse and its later study in the laboratory is a useful diagnosis tool for the veterinarian. In the past several years, an increase of slaughtered young boars due to bad semen quality has been recorded. This fact makes the animal amortization worse and decreases the productive capacity of the boar stud. The aim of this study was to see if there is any link between anatomical, histological study (through in vivo biopsies) and reproductive problem or semen quality able to make an early diagnosis of treatable diseases, thus increasing the sow and boar retention rate.
Materials and Methods: 40 testes from boars and 37 uterus and ovaries from sows under 18 months of age were collected in the slaughterhouse. Boars were culled due to the bad quality of their ejaculates, disease or genetic progress. Sows were culled due to disease or absence of heat. The following parameters were assessed: macroscopic examination, histological evaluation and testes biopsy.
Results: From the 40 boars, 85% were slaughtered due to bad semen quality (abnormal forms, or low volume/concentration) or libido, 5% due to lameness and 10 % due to genetic progress. In 90% of cases, macroscopic lesions were identified. Most common lesions were oedema, inflammation, fibrosis and varicocele. The epididymis was the most frequently injured area. The microscopic study of injuries found is essential to confirm the macroscopic diagnosis. From the 37 sows, the most common ovarian pathology is the anoestrus, many times ovaries are cyclic and it is due to a management failure related with the heat detection. The most frequent pathology in the correct discarded sows is the endometritis and it can be related with salpingitis.
Conclusion: Our results confirm that boars were properly culled and that mainly lesions were chronic and diffuse. The impossibility of performing epididymal biopsies would focus the effectiveness on a testicular level, losing diagnostic sensitivity. Many of the study results of the sow genitalia in the slaughterhouse can be extrapolated to other sows with the same symptomatology, and allow the recommendation of a therapeutic pattern for other animals affected in the farm or artificial insemination center.

Disclosure of Interest: None Declared

Keywords: slaughterhouse, pathology, genitalia

Reproduction

PO-PW1-250

POST CERVICAL INSEMINATION IN GILTS BY THE USE OF A SPASMOLYTIC

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Introduction: Post Cervical Artificial Insemination (PCAI) is routinely used by many companies. But there is a problem, PCAI is generally used in multiparous sows, because when you try to apply it in gilts, in about 50% of them, the post cervical cannula can't pass through the cervix. It has been suggested that the problem is the lower reproductive tract development in gilts, but the fact that in some sows, in their AI after the first weaning, there are also difficulties passing the cannula seems to override this theory.
During PCAI the catheter is captured by pressure of the cervix and a later state of relaxation is necessary to permit the passage of post cervical cannula. We think that due to fear and nervousness, gilts need more time to relax, and in some cases the right level of relaxation can't be achieved because the physiological contractions turn into spasm.
This paper tries to test out if it is possible to eliminate the spasm of the cervix by using a spasmolytic, allowing the passage of the cannula and therefore the PCAI, without altering the normal pattern of uterine contractility.
Materials and Methods: The trial was carried out in a sow farm, in which PCAI is used in weaned sows in a habitual way. A batch of gilts in oestrus, with 135 kg and 235 days of age, previously synchronized with Altrenogest, were randomly divided in two groups, Hyoscine (H) and Control (C). In group H, 12 gilts were injected with 20 mg (1ml) of Hyoscine butylbromide and in group C another 12 gilts served as control and were inseminated in a traditional way. In group H, gilts were injected subcutaneously 10 minutes before starting PCAI in the groove formed between the outer face of the vulvar lip and the ham in order to reach the irrigation area of branches from the vaginal artery that anastomose with the descending branches of the uterine artery.
For PCAI in group H and for standard AI in group C, 45 ml and 90 ml doses were used respectively.
Results: All sows in both groups were inseminated 3 times, 24-hr interval. No signs of pain were observed while were injected. Farrowing rate was 83.33% in both groups. Litter size was very similar, 12.30 total born and 11.40 born alive in group H and, 12.20 total born and 11.70 born alive in group C.
Conclusion: Although the number of gilts on the trial is small, the fact that has been possible to inseminate all treated sows with spasmolytic, 36 inseminations in total, suggests that the spasm is responsible for the failure to perform PCAI in gilts.
Likewise, the absolutely normal parameters in fertility and prolificacy also seem to show that the elimination of spasm in the cervix don't affect the normal behaviour of the uterus

Disclosure of Interest: None Declared

Keywords: Gilts, POST CERVICAL INSEMINATION , SPASMOLYTIC

Reproduction

PO-PW1-245

Synchronisation of gilts by an altrenogest oral solution in a Korean herd

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Introduction: Altrenogest is used worldwide to synchronise sexual cycle of replacement gilts before their introduction in the reproductive herd. Objective of this study was to test efficacy of a 0.4% altrenogest oral solution (Virbagest®, Virbac) for gilts synchronization in a large Korean farm.
Materials and Methods: Selected herd comprises 2,500 sows managed according to 1-week batch farrowing. Twenty to 30 replacement gilts are introduced weekly in each batch. Target is to service 240 days old gilts weighing around 140 kg. Two hundred seventy cycled gilts were included in summer during 10 successive weeks per batch of 25 to 30 each. The altrenogest oral solution was given orally for 18 days at the dose of 18 mg/d. Oestrus was detected after stop of treatment by boar stimulation twice daily. Gilts showing standing reaction were serviced twice 24 h apart by artificial insemination. Pregnancy was diagnosed twice by ultrasonography (27 and 34 days after service). Regular and irregular returns were checked till 7 weeks after service. The 95% confidence intervals (CI) were calculated for rate of gilts showing oestrus and pregnancy rate.
Results: Out of the 270 treated gilts, 262 showed oestrus and were serviced (97.0%, CI = 95.0% - 99.0%). Grouping of oestrus was obtained within 3 days for 99.2% of serviced gilts (6 to 8 days after stop of treatment). Pregnancy rate among serviced gilts was equal to 95.0% (CI = 92.4% - 97.6%). Reproductive performances of the treated gilts will be followed till weaning of first litter.
Conclusion: This study confirmed the accurate grouping of gilts oestrus following treatment with the tested altrenogest oral solution. Such synchronisation allows an anticipated introduction of replacement gilts whatever the type of batch management, with well known advantages (optimisation of the number of required gilts and selection of them at the right time, stability of replacement rate over time, heat detection focused on a few days).

Disclosure of Interest: None Declared

Keywords: altrenogest, Gilts, synchronisation

Poster Abstracts

Reproduction

PO-PW1-225

Acute phase protein and cytokine levels in serum of sows during the periparturient period

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Introduction: A considerable number of modern pig herds suffer from problems with postpartum dysgalactia syndrome (PDS). PDS is a syndrome with a complex pathophysiology and several risk factors involved, thus the proper prophylaxis and diagnosis is difficult. During last years an increase of interest in porcine acute phase proteins (APP) and cytokines regarding their potential use as biomarkers has been observed. However, the knowledge about their serum concentrations during the physiological and pathological periparturient period in sows is restricted. Therefore, the aim of the study was to determine the serum concentration of selected APP and cytokines in sows around parturition. The utility of mentioned proteins in the early diagnosis of PDS was also investigated.
Materials and Methods: One hundred thirty nine sows from 2 herds were used. Based on the course of the periparturient period (day -28 to +28) females were divided into 3 groups (A: healthy n=58; B: PDS n=45; C: other (lameness, injuries, difficult parturition without PDS, etc. n=36). Pigs were bled at -28 (-25-30), -14 (-16-11), -7 (-8-6), -3, -1, 0 (parturition), +1, +3, +7, +14, +28 days of study. The concentration of CRP, Hp, SAA, Pig-MAP, IL-6, IL-8 and TNF-a were examined using a commercial, pig-specific ELISA assays. The production and clinical parameters were also recorded.
Results: The significant increase of investigated APP, IL-6 and TNF-a concentration has been observed in healthy females after farrowing ($p<0.05$), while level of IL-8 remained unchanged ($p>0.05$) as compared to the level observed at days 28 and 14 prepartum. In pigs from group B the concentration of SAA and Pig-MAP were significantly higher 7 and 3 days before farrowing as compared to females from groups A and C ($p<0.05$). The concentration of IL-6, TNF-alfa, CRP, Hp, SAA and Pig-MAP at various time point postpartum also differed significantly in females from various groups ($p<0.05$). We found the significant positive correlation between concentration of SAA and Pig-MAP at days -7 and -3 of study and PDS severity (R-Spearman ≥0.64, $p<0.05$).
Conclusion: Results of the present study revealed the significant changes in the concentration of APP, IL-6 and TNF-a around normal parturition. After farrowing, the level of cytokines and APP was generally higher in sows with PDS then in healthy females. The significant correlation found between concentration of SAA and Pig-MAP before farrowing and PDS severity indicate their potential utility as early biomarkers of PDS in sows. The further studies should be undertaken to investigate whether early intervention (i.e. antibiotic and/or anti-inflammatory treatment) may prevent clinical manifestation of PDS.

Disclosure of Interest: None Declared

Keywords: biomarkers, PDS, periparturient period

Reproduction

PO-PW1-244

SYSTEMATIC USE OF MONZAL® IN FARROWING SOWS REDUCES STILLBORN PIGLETS AND IMPROVES THE HERD PROFITABILITY

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Introduction: Incidence of stillbirths is a major cause of piglet loss before weaning.
The use of vetrabutine or oxytocin seems to be interesting in order to decrease the duration of piglet expulsion (1). Nevertheless the use of oxytocin may increase intraparturition piglet mortality (2).
The objective of this study was to evaluate the reduction of stillbirths implementing the use of vetrabutine (Monzal®, Boehringer Ingelheim Vetmedica GmbH) systematically in hyperprolific sows at farrowing.
Materials and Methods: The study was conducted in a 1200 hyperprolific sows farm in Spain.
Since February 14th until April 4th 2014 overall 428 sows were randomly selected for this study. The control untreated group included 274 sows. In the treatment group 154 sows were injected with Monzal® after the first born (3ml/gilts and 4ml/multiparity sows). No prostaglandin treatment was applied.
Data collected were farrowing parameters such as total born, live born and stillborn piglets.
The data were evaluated by Analysis of Variance (ANOVA) test procedures using SPSS v.15.0 software (SPSS Inc., Chicago, IL, USA).
The return on investment was calculated using a large economics data base (3) and market prices for the product.
Results: Table 1 reveals that, total born piglets was similar for both treatments (+0,14 for control group). The Monzal® group had 0,69 stillbirth less than the control group with statistical difference. Thus sows treated with Monzal® had more born alive piglets (12.13) than those from the not treated control group (11.58).In addition the variation of the stillborn parameter was also reduced in the Monzal group ($p<0.001$).When using all of parameters including improvements at farrowing and reduction of stillbirths, in an economical tool calculator, Monzal group showed a large return on investment of 8,98€ per euro invested.
Conclusion: This study has demostrated that the systematic use of Monzal® improves sow herd performance parameters.
In hyperprolific sows the use of Monzal® in the beginning of farrowing has shown a reduction of stillbirths, particularly for the high stillborn litters and therefore an increase of the number of weaned piglets per sow/year.
As it has been previously shown (4), the use of Monzal® increases the profitability of the sow herd.

Disclosure of Interest: None Declared

Keywords: Monzal, performance improvement, Stillborn reduction

Reproduction

PO-PW1-251

Comparison of PRRS MLV type2 vaccine efficacy of Pre-farrow vaccination program and Mass vaccination program in Thai swine farm

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Introduction: PRRSv continues to challenge pig producers in Thailand and many countries. Herd closure associated with exposure to live attenuated vaccine to stabilize herd is the common practice. The aim of this study was to investigate the 2 different PRRS control strategies and its impact on sow performance in the breeding herd.
Materials and Methods: The observation of this retrospective study was conducted in 3,500 sows, farrow to finish herd in a high density area. The herd applied VR2332- based vaccine as part of a PRRSv control strategies. During 2012 - 2013 P0-P2 Unit implement PRRS MLV vaccine as Pre-farrow program at 10 week of pregnancy stage. At the end of 2013, the P0-P2 Unit had a high percentage of illness sows during gestation and farrowing period. In December of 2013, PRRS virus was detected in the samples that taken from late term pregnancy sows by PCR method. After that, the P0-P2 Unit changed to the quarterly mass vaccination program through year 2015. The P3-P6 Unit has been on a quarterly mass vaccination program throughout the study.
Results: The overall sow performances have increased in the mass vaccination method period. The farrowing rate has increased from 78% to 86%. The % Stillborn, % Mummified and % Pre-weaning mortality has decreased respectively (9.58 vs 6.96, 2.75 vs 0.95 and 17.3 vs 10.5)
Conclusion: Consistent implementation of strategic sow herd mass vaccination program using Ingelvac PRRS MLV vaccine for control of PRRSv infections was effective in improving sow performance and reducing pre-weaning mortality. When vaccinating at day 80 of pregnancy, there is a high risk of creating subpopulations of sows, that has different duration between vaccinations. All sows that have returned to oestrus would have longer times between vaccinations. In addition to the vaccination at day 80 of pregnancy could be a risky strategy. With mass vaccination, it is ensured that all sows have equal immunity in the breeding herd, at all timepoints. This has shown to be the most optimal strategy to ensure the most beneficial reproductive performance.

Disclosure of Interest: None Declared

Keywords: farrowing rate, mass vaccination, pregnancy

Reproduction

PO-PW1-214

Blood glucose predicts survival in piglets

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Introduction: Piglet mortality causes welfare challenges and economic losses in commercial pig production. Crushing is the most common cause of death, but as an empty stomach is frequently found in crushed piglets, starvation is often a precursor to be crushed. Piglets are born with a limited resource of energy in the form of glycogen and with very little body fat. Thus an abundant amount of sow colostrum and later sows milk is vital for the survival of the piglets. In most published studies the mortality in small piglets (600-1000 gram) is 20- 30 %.
Materials and Methods: The study was performed in two commercial sow herds using DanAvl DxLY genetics. When the manager expected that the piglets had obtained colostrum, the smallest piglets were observed for a few seconds to see, if they had a teat or not. Piglets lying under the heat lamp were activated before observation. The piglets were weighed, and 158 piglets weighing below 1050 gram were included in the study and eartagged. Blood glucose was measured using an onsite device (Accu-Check Aviva (www.MediqDanmark.dk)) after venipuncture. Rectal temperature was measured using a digital thermometer. The small piglets were then transferred to nurse sows, nursing only small piglets, and kept under routine management until outcome was registered at day 14. For control, 30 piglets weighing between 1200 and 1600 gram were tested for blood glucose. These 30 piglets were not followed any further.
Results: The limit for hypoglycemia was set at 2.8 mmol/liter. None of the 30 control piglets had levels of blood glucose below 2.8 mmol/liter. Among the 158 small piglets, 38 % showed hypoglycemia. Small piglets with blood glucose above 2.8 mmol/liter had just 6 % mortality, while small piglets hawing hypoglycemia had 35 % mortality. Hypoglycemia at the time of adjustment of litter size is a good indicator of piglet mortality. Thirty of the 158 piglets were not actively nursing at collection. Of these, 26 piglets had hypoglycemia (87 %). Among the 128 piglets with normal activity, 34 piglets had hypoglycemia (27 %). Mortality was 18 % if piglets had a temperature above 37^0C (155 piglets), and 44 % if temperature was 37^0C or below (22 piglets).
Conclusion: Normal blood glucose in a small piglet indicates a good chance to survive. Of piglets with hypoglycemia shortly after birth, 36 % died before the last observation on day 14. Hypoglycemia was thus a good predictor of piglets dying after adjustment of litter size. Not having a specific teat was a good predictor of hypoglycemia. Finding causes for the piglets suffering hypoglycemia is an important step in reducing piglet mortality.

Disclosure of Interest: None Declared

Keywords: Piglet survival glucose

Poster Abstracts

Reproduction

PO-PW1-206

IMPROVEMENT OF PRODUCTIVITY EFFICIENCY IN LARGE SIZE FARMS THAT USE PORCEPTAL® IN A FTI PROGRAM

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Introduction: Spanish swine production systems have changed as farms are becoming larger. Reproductive management efficiency, productivity and production costs become more important. The aim of this study was to demonstrate feasibility of implementing a systematic approach for breeding weaned sows by inducing ovulation and to confirm efficacy and profitability of ovulation induced with Buserelin (Porceptal®) followed by single fixed time insemination (FTI) of sows.
Materials and Methods: The trial was done in a 3000 sow farm with 3 phase production and located in the center of Spain. It was conducted in February 2015 and included a full weaning, 65 sows in control group and 45 sows in the Porceptal® group. The sows were randomized in both groups taking into account cycle, body condition, and previous weaning. The control group followed standard management procedures for estrus check and insemination. The Porceptal® group sows were injected with 2.5 ml Porceptal® i.m, 83-89 h post-weaning. About 30-33 days later, sows were checked for heat, and if positive, were inseminated with one dose of commercial semen (FTI). Both groups used post-cervical artificial insemination. Reproductive data, such as fertility and prolificacy, were compared via Fisher Test, Pearson Chi Square Test and Levene Test and ANOVA.
Results: In Porceptal group, 95.6% of sows were in heat and used FTI program five days after weaning, while only 83% of control sows were inseminated before 7 days post-weaning. The average weaning to mating interval was 5 days in P vs 7.03 in control group (p=0, 003). Farrowing index was not statistically different between study groups (86.2% C vs 83.7% in P, p=0.945). Average gestation length was 114.82 in control group and 114.17 in P group (p=0.022), 26.6% had 116-117 days in control group, vs only 11% sows in Porceptal group. The total piglets born were 14.11 C vs 14.78 P (p=0.319), alive 13.34 C vs 13.75 P (p=0.648). Return on investment was 2.0, representing 35.474 € in annual benefits.
Conclusion: Porceptal could be a very useful tool for FTI programs in large farms, considering that fertility and prolificacy is similar to standard multiple inseminations programs. However, FTI results in semen savings and other benefits such as farrowing groupings, reduction of weaning to mating interval, reduction of non-productive days and efficiency in the organization and management of the farm.

Disclosure of Interest: None Declared

Keywords: Porceptal, FTI, productivity

Reproduction

PO-PW1-221

BIRTH WEIGHTS IMPROVE AFTER CONTINUED USE OF PORCEPTAL IN A COMMERCIAL FARM

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Introduction: Results of multiple studies demonstrated the benefits of buserelin, a GnRH analogue, in Fixed Time Insemination (FTI) programs. Benefits include synchronized ovulation, more efficient use of workforce, better grouping at time of farrowing, and reduction of semen doses. The objective of this study was to confirm these benefits with continued use of Porceptal® (buserelin, MSD Animal Health), in multiparous sows and also evaluate effectiveness in ovulation induction and influence on piglet's birth weight
Materials and Methods: The trial was conducted in a 250 sow farm in Northern Spain (Aragon). A total of 400 weaned sows were included in this study between Apr 2015 and Nov 2015. A comparison was done between the months prior to introduction of Porceptal and after. Sows that were not included in the Porceptal Group (primiparous and sows that came in heat before treatment mainly), group C, were compared against test sows that participated in the full FTI period, group P. P group was treated with Porceptal 83-89h after weaning and was inseminated 30-33h later. Control group followed the standard farm insemination protocol. In both groups, post-cervical artificial insemination was applied. Fertility and prolificacy, semen doses and weights at birth data were recorded. Reproductive data and birth weight were compared with Mann-Whitney U Test of Levene and ANOVA
Results: Fertility was not statistically different between study groups, C 75.2% vs P 83.3% (p = 0.093). Total Born, P 11.8 vs C 12; (p > 0.05) were also not statistically different. A total of 564 birth weights from Control and 1084 from Porceptal, originating from 139 litters (47 control, 92 Porceptal) were included in the analysis. In Group C, the mean birth weight was 1.43 kg (1.39-1.46 confidence interval, median 1.45). In P group average weight was 1.49 kg (interval of confidence 1.47 to 1.51), medium 1.50. Coefficient of variation (CV) was in C = 0, 2659 and P = 0, 2393. These results were significantly different between groups (p = 0.008). One sperm dose per sow was used in the P Group, compared to an average of 2.6 doses per sow in the conventional protocol
Conclusion: Fertility and prolificacy results were similar during the period that Porceptal was used and before. A single semen dose compared to 2.6 in control sows reduced production costs and resulted in fewer boars cycle in the FTI program. Both aspects improve workforce efficiency and grouping at time of farrowing. Lastly, birth weights were significantly higher and coefficient of variation was lower during the Porceptal® study period. Impact of improved birth weight on overall growth remains to be investigated

Disclosure of Interest: None Declared

Keywords: Porceptal, FTI, semen

Reproduction

PO-PW1-220

ECONOMICS OF SINGLE FIXED TIME INSEMINATION PROTOCOL IN A COMMERCIAL SPANISH FARM

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Introduction: GnRH analogues as buserelin are able to induce an LH release to synchronize ovulation, allowing single Fixed Time Insemination (FTI). The aim of this study was to demonstrate that a single FTI protocol following buserelin injection (Porceptal®, MSD Animal Health) generates similar reproductive performance as the one observed in conventional management with multiple inseminations.
Materials and Methods: The trial was conducted in a 400 sow farm in Catalonia. A total of 158 sows, weaned between 23Jul14 to 24Sep14, were randomly distributed in two groups based on productive cycle: Control (C; 67 sows) and Porceptal (P; 91 sows). From weaning to Porceptal® injection, all sows were managed the same. The same boars and same personnel inseminated and checked heat in the two groups. Control sows were inseminated following the standard farm insemination protocol. Porceptal® sows were injected with 2.5ml Porceptal® i.m (4µg Busereline/ml), 88-89h post-weaning. About 31-32 hours later, sows were checked for heat, and if positive, were inseminated with one dose of commercial semen. No heat detection was done after this one and only insemination. Reproductive data, such as fertility and prolificacy, were compared via Fisher Test, Pearson Chi Square Test, Levene Test and ANOVA.
Results: All P sows showed estrus after weaning, while 3% of C sows were not in estrus. Fertility was not statistically different between study groups (95,3% C vs 92,3% C; p=0,5). Gestation length was lower in P sows (114d P vs 115d C). Total Born (14,79 P vs 14,68 C; p=0,9) and Total Alive (13,61 P vs 12,95 C; p=0,1) were also not statistically different. Nevertheless, although there were no differences in percent farrowings with stillbirths, total stillbirths were significantly lower in the P group (1,18 P vs 1,73 C; p=0,02). Porceptal sows had a higher farrowing concentration, with 58,4% between Wednesday and Friday compared to 45% in Controls. One semen dose per sow was used In the P Group, while the average of the conventional protocol was 2,4 doses per sow
Conclusion: Although both C and P Groups had similar results in terms of fertility and prolificacy, P group clearly had advantages such as workflow organization, reduction of semen doses, concentration of farrowings and reduction of stillbirths. Total ROI (extrapolated to Porceptal use for one year) calculated based on stillbirths, workflow, semen doses and Porceptal cost was 4,3. In an integration company, the ROI would be 2,5 because of the different distribution of costs. Therefore, Porceptal can be considered as an efficacious and profitable alternative to the conventional insemination system.

Disclosure of Interest: None Declared

Keywords: Economics, FTI, Porceptal

Reproduction

PO-PW1-228

The use of Peforelin in gilts and sows under industrial pig farm conditions

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Introduction: Peforelin is the synthetic analogue of the lamprey GnRH-III which is characterized by a distinct selectivity for the release of FSH also in the pig. Various clinical studies confirm its efficacy in inducing oestrus in weaned sows and gilts after synchronization of the sexual cycle. The objective of this GCP-study was to generate experiences with the use of Peforelin under industrial farm conditions.

Materials and Methods: The study was performed in a Polish industry farm with 1800 Polish Large White x Polish Landrace sows, managed in a 1-week-production rhythm with 28 days lactation length. The sexual cycle of the gilts were synchronized by Altrenogest through a period of 18 days. 48 hours after the last Altrenogest treatment the gilts were allocated to one of two groups: Peforelin-Group 1 (n = 40): Gilts were treated with Peforelin. Control-Group 1 (n =42): Gilts untreated. AIs in both groups were performed according to a unified protocol. 24 hours after weaning sows were assigned to Peforelin-Group 2 (primiparous sows, n = 60), Peforelin-Group 3 (multiparous sows, n= 61) or to Control-Group 2 (untreated primiparous sows, n = 60) or to Control-Group 3 (untreated multiparous sows, n = 60). AIs were performed in Peforelin- and Control-Groups according to a unified protocol. To assess the reproduction performance oestrus events and farrowing results were recorded. Statistical analysis was made by the use of ANOVA, statistical differences between analysed means were determined depending on number of analysed groups by Duncan or Tukey test.

Results: The highest Non-Return-Rate was observed in Peforelin-Group 1 and 3 ($p \leq 0,05$). A pronounced effect of Peforelin was observed in farrowing rate. The biggest difference was between gilts and multiparous sows, respectively 6,4 % and 4,9 % ($p \leq 0,05$). There were no statistically confirmed differences between groups in number of total born and live born piglets. The highest Piglet Index was observed in Peforelin Group 1 and 3. The difference was respectively 64 and 76 live born piglets/100 AIs vs. Control Group 1 and 3.

Conclusion: According to the collected data the use of Peforelin under industrial farm conditions results in improving the reproduction performance of production batches in gilts after the synchronization the sexual cycle and multiparous sows. This notice is based on higher farrowing rates. The reproduction performance of the individual sow was not influenced. In consequence an overstress ("fertility doping") of the sows can be precluded. Further investigations for the use of Peforelin in primiparous sows are recommended.

Disclosure of Interest: None Declared

Keywords: Batch management system, Peforelin, Reproduction Performance

Poster Abstracts

Reproduction

PO-PW1-236

Induction of parturition by using a single or split-dose of PGF2alpha in sows

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Introduction: Induction of parturition by using prostaglandin F_{2a} (PGF_{2a}) is frequently used in swine commercial herds worldwide. In practice, different protocols have been implemented. The benefit of induction of parturition in sows in combination with farrowing supervision includes decrease the risk of stillborn piglets, decrease postpartum complications in sows and decrease the incidence of post-partum dysgalactia syndrome. However, if the induction of parturition protocol is not properly applied, unpredictable farrowing may be occurred. The aim of the present study was to investigate the effect of induce parturition by using a single-dose of PGF_{2a} or a repeated injection of PGF_{2a} (split-dose) on the interval from the hormone injection to farrowing.
Materials and Methods: The experiment was performed in a 1500-sow commercial swine herds in Thailand. In total, 91 Landrace x Yorkshire crossbred sows were included in the experiment. The animals were randomly assigned into one of three treatment groups: control, single-dose and split-dose of PGF_{2a}. The hormone was administrate in the intra-vulvo-submucosa route by using a synthetic analog of PGF_{2a} (0.087 mg Cloprostenol Sodium, 1 mL Planate®; MSD Animal Health, USA). In the treatment groups, PGF_{2a} was administered at 8:00 AM on day 114 of gestation (single-dose) or at 8:00 AM and 2:00 PM on day 114 of gestation (split-dose). In the control group, sows were allowed to farrow naturally. The onset of farrowing was monitored 24 h and the duration of farrowing was also recorded. The time interval from PGF_{2a} to farrowing was calculated and presented as frequency distribution.
Results: The percentage of stillborn piglets (2.9% versus 5.6% and 6.7%) and the incidence of dystocia (27% versus 54% and 42%) in the sows induced parturition by using split-dose of PGF_{2a} tended to be lower than single-dose and control groups, respectively. Of the sows induced parturition by using split-dose of PGF_2, 43.5% and 56.0% farrowed within 8-22 h and 23-33 h after the first PGF_{2a} administration, respectively. Of the sows induced parturition by using a single-dose of PGF_{2a}, 28.1%, 62.5%, 3.1% and 6.3% farrowed within 8-22 h, 23-33 h, 34-46 h and >47 h, respectively. In the control group, 15.4%, 7.7%. 23.1% and 30.8% of the sows farrowed within 8-22 h, 23-33 h, 34-46 h and >47 h after the onset of treatment, respectively. Split–dose of PGF_{2a} reduced the variation of farrowing time compared to the single-dose and control groups.
Conclusion: Farrowing induction at 114 days gestation by using split-dose of PGF_{2a} improve the efficacy of farrowing management by reducing variability of the farrowing times.

Disclosure of Interest: None Declared

Keywords: Farrowing, Predictability, Postpartum

Reproduction

PO-PW1-237

Colostrum yield in sows after induced parturition by using PGF2alpha

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Introduction: Colostrum is the first milk secreted by the mammary gland of the sow from 0 up to 12–24 h postpartum. Colostrum is a source of digestible nutrients and various compounds, e.g., immunoglobulins, hormones and growth factors. Thus, it plays a key role in piglet thermoregulation, the acquisition of passive immunity and intestinal development. Induction of parturition by using prostaglandin F_{2a} (PGF_{2a}) is frequently used in swine commercial herds worldwide. The aim of the present study was to investigate the influence of induce parturition on the colostrum yield in sows.
Materials and Methods: The experiment was performed in a 1500-sows commercial swine herds in Thailand. In total, 91 Landrace x Yorkshire crossbred sows and 974 newborn piglets were included in the experiment. The animals were randomly assigned into one of three groups: control (natural farrowing), and treatment with a single- and split-dose of PGF_{2a}. The hormone was administrated in the intra-vulvo-submucosa route by using a synthetic analog of PGF_{2a} (0.087 mg Cloprostenol Sodium, 1 mL, MSD, USA). In the treatment groups, PGF_{2a} was administered at 8:00 AM on day 114 of gestation (single-dose) or at 8:00 AM and 2:00 PM on day 114 of gestation (split-dose). Farrowing process was monitored 24 h. The newborn piglets were weighed with a digital balance immediately after birth and again at 24 h after birth. Colostrum intake (gram) of the piglets = [-217.4+(0.217*t)+(1,861,019*BW24h/t)+BW*(54.8-1,861,019/t)*((0.99853.7 $*10^{-4}$*tFS)+(6.1*10^{-7}*tFS^2)]; where BW=birth weight (kg), BW24h=body weight at 24 h after birth (kg), t= time elapsed between the first and the second weighting (min), and tFS = the interval between birth and first sucking (min). Colostrum yield in sows was defined as the sum of individual piglet's colostrum intake.
Results: The percentage of piglets with umbilical rupture in the sows induced parturition by using split-dose of PGF_{2a} (29.0%) was higher than single-dose (15.0%) and control groups (20.1%) (P<0.05). Colostrum yield of sows induced parturition by using a single dose and split-dose of PGF_{2a} did not differ significantly compared to the control groups in both primiparous (2716, 2853 and 2747 grams, respectively) and multiparous sows (4036, 3612 and 3354 grams, respectively). However, colostrum yield in multiparous sows was higher than primiparous sows (P<0.05).
Conclusion: Farrowing induction at 114 days gestation by using PGF_{2a} did not influence the colostrum yield of the sows. Colostrum yield in multiparous sows was higher than primiparous sows.

Disclosure of Interest: None Declared

Keywords: Colostrum, Farrowing, Sow

Reproduction

PO-PW1-239

Follicle development, ovulation, and evidence of silent heat in gilts after estrus induction using gonadotrophin under tropical climate

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Introduction: Gonadotrophin is a hormone practically used for induction of estrus in gilts and sows. In pigs, exogenous gonadotrophin commonly used contains a combination of 400 IU eCG and 200 IU hCG. In general, the hormone is recommended to use in gilts with ≥85 kg body weight and 165 days of age. Previous studies found that 65–100% of the gilts ovulated within 10 days after treatment, but up to 30% do not exhibit behavioral estrus. The objective of the present study was to determine follicle development, vulva symptoms, ovulation and evidence of silent heat in gilts response to gonadotrophin treatment under tropical climate.
Materials and Methods: The present study was carried out in a 2500-sow commercial herd in Thailand. In total, 65 Landrace x Yorkshire crossbred gilts were included in the experiment. The gilts were weighed and treated with a single dose of gonadotrophin (PG600®, MSD, NJ, USA) at 188 ± 10.6 days of age. Estrus behavior was detected twice a day using a fenceline exposure to 2 mature boars. Vulva symptoms (i.e., reddening and swelling) were carefully determined daily. The vulva symptoms were classified as 0 (no vulva symptoms) or 1 (mild to strong vulva symptoms). The gilts were slaughtered at 3 (n=30) and 7 days (n=35) after gonadotrophin treatment. Numbers of medium size (3-6.5 mm) and large size follicles (>6.5 mm) and number of corpora lutea were counted. The data were analyzed using descriptive statistics.
Results: On average, the age, body weight, backfat thickness and average daily gain of the gilts were 188 days, 99.5 kg, 7.8 mm and 532 g/day, respectively. The percentages of gilts that exhibited standing estrus were 0% and 48.6% at 3 and 7 days after treatment, respectively. Number of medium and large size follicles at 3 days were 10.3 and 13.7, respectively. Ovulation occurred in 6.7% and 65.7% of the gilts at 3 and 7 days after treatment, respectively. Vulva symptoms (score of 1) were observed in 46.2%, 70.8%, 76.9%, 71.4% and 57.1% of the gilts on Days 1, 2, 3, 4 and 5 after treatment, respectively. The present study indicated that the estrus behavior of the gilts after gonadotrophin treatment in tropical climates is relatively low (48.6%). Nevertheless, ovulation occurred in 65.7% of the gilts.
Conclusion: The present study indicates that silent heat was detected in 17.1% (6/35) of the gilts within 7 days after gonadotrophin treatment. The gilts can be classified into three groups, i.e., no response at all (34.3%), silent heat (17.1%) and response (48.6%). Therefore, both internal and external factors associated with the estrus behavioral response after gonadotrophin treatment in gilts under tropical climates should be investigated further.

Disclosure of Interest: None Declared

Keywords: None

Reproduction

PO-PC01-018

Use of OvuGel® Improves Pigs Born Alive per 100 Sows Weaned and Increases Average Age of Pigs Weaned

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Introduction: OvuGel® (OG) contains a GnRH agonist, which stimulates LH release causing ovulation 40-48 hours later. OG is administered intravaginally 96 hours post-weaning followed by a single insemination (AI) 24 hours later without regard to estrus. This study was conducted to determine if OG would improve the number of weaned sows that subsequently farrow, and increase the age at weaning within a farrowing group.
Materials and Methods: A total of 833 sows at a 7,000 sow commercial farm were allocated to this study. Controls were AI twice at detected estrus (n = 426). Treated sows received OG 4 days after weaning and were AI once 23-24 hours later (n = 407). A boar was present during estrous detection. All OG sows were mated without regard to estrus, but estrous status was recorded at time of mating.
Results: Ninety three percent of the Control sows were in estrus and inseminated multiple times by day 7 post-weaning and 93% of the OG sows were in estrus at the time of single fixed- time AI on day 5 post-weaning. The OG sows inseminated without expressing estrus had a 39% farrowing rate, resulting in more OG weaned sows farrowed compared to the Controls (88.3% *vs* 84.6%, respectively; $P = 0.119$). Number of pigs born alive was similar between treatments ($P = 0.82$), but more OG sows farrowed than Controls. Therefore, there were more total pigs born alive per 100 OG weaned sows than for Controls (1075 *vs* 1039). When all weaned sows that eventually farrowed were included in the analysis (Control sows bred after day 7 post-weaning and recycled sows that were rebred in both treatment groups), the farrowing rate was 88.7% for Control and 94.6% for OG sows ($P < 0.46$). The number of pigs born alive per 100 weaned Control sows was 1089 compared to 1166 for OG sows, resulting in a 77 pig advantage for the OG sows. OG sows required fewer ($P < 0.01$) semen doses per successful mating and had 1.2 additional days of lactation, resulting in an older pig at weaning ($P < 0.01$). The use of OG contributed to more weaned sows farrowing, reduced non-productive days (sows not in estrus that farrowed), increased wean age and reduced semen doses for mating. The net financial effect was modeled on value drivers selected by the producer and included semen cost savings, reduced non-productive days, increased genetic merit and wean age improvement. This produced a conservative annualized return over investment of $14.59 per sow and a full value return of $45.37 per sow if extra pigs produced were included in the model
Conclusion: The use of OvuGel® to synchronize mating added significant value in this production system.

Disclosure of Interest: None Declared

Keywords: OvuGel, Pigs, Sows

Poster Abstracts

Reproduction

PO-PW1-226

SEMINAL PLASMA FROM THE SPERM-RICH FRACTION INFLUENCE MEMBRANE INTEGRITY OF EXTENDED LIQUID BOAR SPERM

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Introduction: Seminal plasma (SP), the non-cellular component of semen, is a complex mixture of secretions produced by different structures of the male reproductive tract and owing to these different sites of production, SP is a heterogeneous fluid composed of several organic and inorganic constituents such as ions, lipids, hormones and various proteins which influence greatly the sperm cell biology. Notwithstanding, SP, under *in vitro* condition, can play an ambiguous role on porcine sperm physiology, acting at the same time beneficially and detrimentally and some studies have regarded SP as not being an adequate medium for sperm storage. Hence, the objective of the present study was to evaluate the effect of seminal plasma, from the rich fraction of the ejaculate on plasma and acrosome membrane integrity and membrane fluidity (capacitation) of liquid extended boar sperm stored at 17° C for 72 hours
Materials and Methods: Four sperm-rich fractions from each of six boars were used. All ejaculates were extended in BTS (30 x 10^6 spermatozoa/mL) and then assigned to one of three treatments, as follows: non-washed seminal plasma (NWSP), centrifuged with own seminal plasma suspended (CWSP) and washed-seminal plasma (WSP). All treatments were held at room temperature for 90 minutes before being evaluated by flow cytometry at 0, 24, 48, 72h. For the simultaneous assessment of plasma and acrosome membrane integrity the following fluorescent probes Hoechst 33342, Propidium Iodide and FITC-PSA were used. For the evaluation of membrane fluidity (Capacitation) the fluorescent probe Merocyanine 540 was used.
Results: Since the time X treatment interaction for all variables were not significant, main effect were analyzed separately. Here we only show the results of main effect treatment. The storage of boar semen in the absence of SP led to a reduction ($P < 0.05$) in the population of sperm with intact plasma membrane and intact acrosome (NWSP, 70.06 ± 0.86^a; CWSP, 69.19 ± 0.88^a; WSP, 53.63 ± 0.96^b%). Regarding membrane fluidity (capacitation), the intensity of fluorescence emitted by the Merocianine 540 probe did not differ ($P > 0.05$) irrespective of treatment (NWSP, 40607.00 ± 2219.90; CWSP, 39517.75 ± 1648.86; WSP, 39432.07 ± 1542.90 a.u.). It should be pointed out that there was no effect of centrifugation ($P > 0.05$) to any of the variables evaluated in the present trial.
Conclusion: SP from the sperm-rich fraction plays a crucial role on the maintenance of a high percentage of sperm cells with intact plasma membrane and intact acrosome, therefore, should be present in seminal doses during liquid storage.

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Disclosure of Interest: None Declared

Keywords: Membrane integrity, Seminal Plasma

Reproduction

PO-PCO1-020

Characteristics of repeat-breeding female pigs on southern EU commercial farms

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Introduction: A repeat-breeding (RB) occurrence increases non-productive days of female pigs (NPD), and consequently decreases herd productivity. However, characteristics of RB female pigs are not well defined or studied in swine. Also, few studies have compared lifetime reproductive performance between RB and non-RB female pigs. Our objectives were 1) to define and characterize RB occurrences using data from commercial farms in southern EU, 2) to examine factors associated with the RB risk, and 3) to assess the reproductive performances of the RB or non-RB females.
Materials and Methods: The data included 121,103 lifetime records and 645,103 service records of female pigs on 125 farms between 2008 and 2013. Applying the definition of RB in cattle to female pigs, an RB female pig was defined as a pig that had had three or more returns, or a pig that was culled due to reproductive failure after its second return within the same parity. The farms were classified into high-, intermediate- and low-performing farms on the basis of the upper and lower 10^{th} percentile of the farm means of annualized lifetime pigs weaned per sow. Multilevel generalized linear models with random intercept were applied to the data. A chi-square test was also used to compare the frequency distributions (%).
Results: Mean RB risks per service for female pigs (± SEM) was 0.5 ± 0.01%. Risks of RB in parities 0, 1 and 2 female pigs were 0.8, 0.5 and 0.4%, respectively, whereas risks of RB in parity 3 or later were only 0.2-0.3%. The RB female pigs had more regular returns, of 18-24 days post service, than non-RB female pigs ($P < 0.05$). Of 3,497 first re-service records of RB female pigs, 47% had regular returns. They also had increased lifetime NPD, ranging from 171 to 206 days, compared with only 78-84 days for non-RB females. Risk factors for RB pigs were low parity (i.e., 0 and 1), summer servicing, farrowing fewer number of pigs born alive and being in low-performing herds. However, gilt age at-first-mating ($P=0.13$), nor number of stillborn piglets ($P=0.64$), nor farm size for females ($P = 0.08$) were associated with RB. For instance, risk of RB in gilts and in summer were 1.5 and 0.8%, respectively, compared to only 0.3 and 0.6% in parity 6 or more and in winter. The RB risks on high-performing and low-performing farms were 0.2 and 2.6%, respectively. The RB females had 55.2-92.5 more lifetime NPD, 1.5-3.3 lower parity at culling and 19.4-39.2 fewer lifetime pigs born alive across parities than non-RB females ($P < 0.05$).
Conclusion: We recommended that producers, especially on low-performing farms, should closely monitor the identified female pig groups at a greater risk of a having RB.

Disclosure of Interest: None Declared

Keywords: farm productivity groups, lifetime performance, repeat-breeding

Reproduction

PO-PW1-213

Benchmarking for reproductive performance and lifetime performance of female pigs on high-performing farms in Southern Europe

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Introduction: Benchmarking has been widely practiced worldwide in the swine industry to improve farm management and productivity. Best practice benchmarking is defined as identifying those practices and processes associated with superior efficiency and performance. In the best-practice benchmarking for breeding farms, measurements in high-performing and ordinary farms have been used to provide values of target performance, using the 10th or 25th upper percentile of the performance measurements as the target values. The objective of the present study was to characterize reproductive and lifetime performance of female pigs on high-performing farms in Southern Europe.
Materials and Methods: The data included 647,498 service and lifetime records of 121,103 females on 125 farms between 2008 and 2013. Annualized lifetime pigs weaned per sow was determined for each sow. Two herd productivity farm categories were defined on the basis of the upper 25th percentile of the herd means of annualized lifetime pigs weaned per sow: high-performing farms (≥ 25.0 pigs) and ordinary farms (< 25.0 pigs). To compare the measurements of female pigs between the two herd productivity groups, two-level mixed-effects logistic regression models and linear mixed-effects models were applied to the data.
Results: Mean values of annualized lifetime pigs weaned per sow in the high-performing and ordinary farms were 24.4 and 20.4 pigs, respectively. For lifetime performance measurements, parity numbers at removal, lifetime pigs born alive and non-productive days on high-performing farms were 4.9, 60.2 pigs and 78.5 days, respectively. The high-performing farms had 0.4 higher parity at removal, 6.8 more lifetime pigs born alive and 25.2 fewer lifetime non-productive days than the ordinary farms. However, there were no differences between the two farm productivity groups for gilt age at-first-mating (P = 0.81) or culling risk due to pregnancy failure (P = 0.65). Farrowing rates and pigs born alive on the high-performing farms were 86.2-91.3% and 11.7-13.0 pigs, respectively. Across all parity groups the high-performing farms had a 5.5-6.5% higher farrowing rates and had 4.2-6.5 more pigs born alive than the ordinary farms (P < 0.05). However, there was no difference between the two farm productivity groups for return intervals (P = 0.14).
Conclusion: Superior lifetime performance measurements on high-performing farms were based on higher farrowing rates, more pigs born alive, better management for reducing non-productive days and lower parity numbers at removal.

Disclosure of Interest: None Declared

Keywords: high-performing farms, lifetime performance, swine

Reproduction

PO-PW1-208

EFFECT OF SEMINAL PLASMA FROM THE SPERM-RICH FRACTION ON KINEMATICS OF LIQUID EXTENDED BOAR SPERM

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Introduction: Seminal plasma (SP), the fluid medium in which spermatozoa are suspended, is a highly complex physiological secretion produced by different structures of the male reproductive tract. Owing to these different sites of production, SP is a rather heterogeneous fluid composed of organic and inorganic compounds such as ions, lipids, hormones and a variety of proteins which play pivotal roles not only on sperm metabolism but also in key events preceding fertilization. Nonetheless, in spite of being crucial for controlling sperm function, SP, *in vitro*, can be deleterious for sperm viability and some studies have regarded it as not being a suitable medium for sperm storage. Therefore, the objective of the present trial was to evaluate the effect of SP, from the rich fraction of the ejaculate on kinematics characteristic of boar sperm stored under refrigeration at 17° C for 72 hours.
Materials and Methods: Four sperm-rich fractions from each of six boars (n=24) were used. All ejaculates were extended in BTS (30 x 10^6 spermatozoa/mL) and then assigned to one of three treatments, as follows: non-washed seminal plasma (NWSP), centrifuged with own seminal plasma suspended (CWSP) and washed-seminal plasma (WSP). The force of centrifugation used was 500x*g*/10 minutes. All treatments were held at room temperature (24° C) for 90 minutes before being transferred to a controlled-temperature incubator and evaluated by the Sperm Class Analyzer (SCA) for the following variables: total and progressive motility, STR, VCL, VAP, VSL, ALH, BCF, LIN and HIPER at 0 (90 min post dilution), 24, 48, 72h.
Results: Since the time X treatment interaction for all variables were not significant, main effect were analyzed separately. Here we only show the results of main effect treatment. The storage of boar semen in the absence of SP was detrimental for sperm motility characteristics since a reduction in total (P < .0001, NWSP, 75.93 ± 0.58^a; CWSP, 75.34 ± 0.54^a; WSP, 63.29 ± 0.88^b%) and progressive motility (P < .0001 NWSP, 62.43 ± 0.84^a; CWSP, 61.36 ± 0.90^a; NWSP, 47.43 ± 0.90^b%) as well as for BFC (P < 0.0045, NWSP, 3.48 ± 0.13^a; CWSP, 3.52 ± 0.13^a; WSP, 2.37 ± 0.05^{b}Hz) was observed in the WSP treatment. As for sperm movement patterns, there was no effect of treatment on VAP (P > 0.05), VSL (P > 0.05), VCL (P > 0.05), STR (P > 0.05) and HIPER (P > 0.05). It is worth mentioning that the process of centrifugation did not influence (P > 0.05) any of the variables evaluated.
Conclusion: SP from the rich fraction exerts a beneficial effect on sperm motility parameters and must be present in seminal doses during liquid storage.

Acknowledgements: FAPESP - 2014/20768-3, 2015/14258-5

Disclosure of Interest: None Declared

Keywords: Seminal Plasma, Sperm-Rich Fraction, Stored Semen

Poster Abstracts

Reproduction

PO-PW1-229

Dietary L-arginine supplementation during late gestation in sows is associated with their backfat loss during lactation

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Introduction: The loss of backfat thickness in sows during lactation period is commonly observed in swine commercial herds under tropical climate. High backfat loss in lactating sows contribute to increase weaning-to-estrus interval. However, increasing the amount of feed during late gestation may cause high backfat thickness at farrowing, and lead to dystocia. Arginine is a precursor for nitric oxide synthesis, an important part for protein, fat and hormone synthesis. Arginine supplementation increases brown adipose tissue and protein synthesis in sows. The present study aims to investigate the effect of L-arginine supplementation in sow's diet during late gestation on relative loss of backfat thickness of sows during lactation.
Materials and Methods: The study was carried out in a commercial swine herd in Thailand. Sows were allocated to four experimental groups: Group I (control), sows were fed a conventional gestation diet; Group II, sows were supplemented with 0.5% L-arginine; Group III, sows were supplemented with 1.0% L-arginine; and Group IV, sows were supplemented with 1.7% L-alanine. The feeding protocols were carried out from week 13 of gestation until farrowing. A total of 166 sows were included in this study. Sow's backfat thickness was measured at the P2 level using A-mode ultrasonography at farrowing and at weaning. Backfat loss and relative loss of backfat (%) was calculated.
Results: On average, the backfat thickness at farrowing were 14.5, 14.5, 13.9 and 15.9 mm in group I to IV, respectively. At weaning, backfat thickness of sows were 11.9, 12.6, 12.2 and 13.8 mm in Group I to IV, respectively. The backfat thickness at farrowing was positively correlated with the backfat loss and the relative backfat loss (r = 0.565 and 0.329, n = 160, respectively; $P < 0.001$). Arginine supplementation significantly influenced the relative backfat loss ($P = 0.047$). On the other hand, parity and interaction between group and parity did not influence the relative backfat loss ($P > 0.05$, respectively). The relative backfat loss in Group III sows (11.8%) was lower than Group I sows (16.9%, $P = 0.019$), but did not differ from Group II (12.6%, $P > 0.05$) and Group IV (13.5%, $P > 0.05$). On average, primiparous sows lost 14.0% of backfat and multiparous sows lost 13.5% of backfat during lactation period.
Conclusion: In conclusion, 1.0% L-arginine supplementation during late gestation is associated with a reduced relative backfat loss during lactation in sows.

Disclosure of Interest: M. Nuntapaitoon Conflict with: Research and researcher industrial Fund, R. Muns: None Declared, P. Tummaruk: None Declared

Keywords: Arginine, sow, relative loss of backfat

Reproduction

PO-PW1-212

Feeding altrenogest in late lactation improves fertility in primiparous sows having reduced litter size

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Introduction: During late lactation ovarian follicles grow and regress in waves and follicular waves have also been documented in pre-pubertal gilts. The wean-to-oestrus interval (WOI) and oocyte quality can each be influenced by when sows are bred relative to stage of the wave. It is known that feeding altrenogest (AT) to primiparous sows after weaning can improve reproductive performances and that ovarian follicle diameters are reported to be greater in sows after AT treatment for 8 or 15 days. These effects likely involve an effect of synchronization of follicle waves with mating. Additionally, it was found that an increase of the number of corpora lutea or progesterone level increases litter size in sows. Sows with small litters (≤8 piglets) may experience a late lactation oestrus or a very short WOI, impairing predictability of return to oestrus after weaning and fertility after breeding. Administration of AT during last week of lactation will prevent lactation oestrus and synchronize oestrus after weaning; this, in turn, may improve subsequent reproductive performance.
Materials and Methods: 40 primiparous Landrace x Yorkshire sows nursing 8 piglets or less at 21 days of a 28 day lactation were included in the study. They were randomly assigned to receive orally 20 mg/d AT (Virbagest®, Virbac) during the last 7 days of lactation (n=20) including day of weaning, or served as controls (n=20). All sows were subjected to transrectal ultrasound ovarian examination at the onset of oestrus and the number of pre-ovulatory follicles (≥0.6 mm.) were recorded. The WOI was recorded and oestrous sows mated within 12 hours after showing standing heat and again 24 hours later by artificial insemination. Subsequent farrowing rates and litter sizes were recorded.
Results: All sows exhibited oestrus and were inseminated. The control group had a shorter WOI than the AT-fed group (2.7±1.0 vs. 5.7±1.4 days, respectively; P≤0.0001). The farrowing rate were not significantly different between treated and control groups (95 vs 90%) reflecting the excellent breeding management on this farm. However, compared to the control sows, the AT-fed sows had more pre-ovulatory follicles (22.7±3.6 vs.17.3±3.5; P≤0.0001), total born piglets (11.9±1.8 vs.10.6±2.0; P=0.03) and piglets born alive (11.2±1.4 vs.9.7±1.9; P=0.01).
Conclusion: The longer WOI in AT-fed sows supports our suggestion that a small litter size will result in abnormally short WOI but that AT will stabilize follicle waves and result in a normal 4-5 day follicular phase and improved oocyte quality. In association with increased follicle numbers, this results in larger second litters.

Disclosure of Interest: None Declared

Keywords: altrenogest, late lactation, litter size

Reproduction

PO-PW1-233

BENEFITS AND IMPLEMENTATION OF PORCEPTAL IN A SPANISH PRODUCTION FARM

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Introduction: Modern pig farms set a mating target based on fertility results of the farm. Therefore, it is crucial that the farrowing objective and the facility use in operations is optimized. Determining the optimal time of insemination is key to achieving adequate fertility and prolificacy and depends on the binomial estrus review - insemination when ovulation, as is known, occurs in the final third of the estrus. The objective of this study was to evaluate Porceptal® (MSD Animal Health), a GnRH analogue, in multiparous sows for effectiveness in ovulation induction
Materials and Methods: This study was conducted in an 800 sow farm, located in Aragon (Spain). A total of 201 multiparous, weaned sows were included in the study and were assigned to a control (C) (105 sows) and Porceptal group (P) (96 sows). All sows were weaned on a Wednesday afternoon; estrus was reviewed in the morning and in the afternoon of the following days until in heat. The P group was treated with 2.5 ml of Porceptal® (10 µgr buserelin), 89 hours after weaning, while the control group was inseminated according to the standard insemination protocol. The Porceptal group only used Fixed Time Insemination 31 hours after treatment with Porceptal®
Results: Sows were randomized relative to cycle number and body condition. The length of estrus was significantly shorter in the P group vs C group, 1.83 vs 2.25 days ($p<0,001$). Sperm doses in the (P) group were reduced compared to (C), 1.02 vs 2.14 ($p<0,001$) while fertility was not significantly different between the (P) group 91.7% and 88.6% group (C) ($p>0,05$).
Prolificacy was also not different. Total born was 12.80 (P) vs 12.49 (C) and was not different ($p=0.390$), while born alive was significantly better in P (12.32) than in C (11.66) ($p=0.029$). The lower number of stillbirths was probably due to a higher farrowing number at the end of the week which resulted in better control and attention (no prostaglandins were used for induction and scheduling of farrowings).

Porceptal® injection allowed FTI which considerably reduced the semen doses in the (P) group, with an average number of piglets born per semen dose at 11.45 in (P) group versus 5.59 in (C) group.
Under the study conditions, Porceptal® would give an ROI of 2.9 € per treatment, when extrapolating the results for a full year production
Conclusion: Porceptal® allowed for synchronized ovulation, more efficient workforce, and better grouping at time of farrowing. This resulted in more homogeneous litters, improved management in the following production phases by grouping the farrowings and better use of elite boars. The total number of piglets born per semen dose used was significantly higher in the Porceptal® group

Disclosure of Interest: None Declared

Keywords: Porceptal, FTI, semen

Poster Abstracts

Residents' ECPHM Session

PO-PW1-261

Abnormal high progesterone levels during farrowing might influence IgG concentration in sow colostrum

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Introduction: Colostrum plays an essential role in piglet survival and growth, providing the piglets with a source for both immunoglobulin (mainly IgG) and energy. The neonatal piglets lack IgG, which makes them dependent on colostrum as the sole source of antibody. Colostrum IgG composition might be highly variable among sows, even though sows are of the same genotype, parity and are reared in a similar environment. Lactogenesis and colostrum production is induced hormonally by a dramatic drop of progesterone (P4) concentrations which leads to a pre-partum peak of prolactin (PRL). There are studies revealing that a severely impaired production of colostrum is related to a delay in the decrease of P4 concentrations during the pre-partum period. Therefore we assumed that there might be an impaired effect of a high level of P4 concentration on quality of colostrum (IgG concentration) during parturition. The aim of the present study was to investigate the potential relationship between abnormally high P4 levels at parturition and poor colostrum IgG content in sows.
Materials and Methods: Blood samples (n=38) were collected from *vena saphena medialis* using ice chilled EDTA K_3 tubes to assess P4 concentration. Samples were centrifuged at 1006 × g for 15 min and the plasma wasfrozen at -20° C until analysis. Two blood samples were collected from each sow, at the beginning and end of farrowing. Colostrum samples (N = 38) were obtained between 0-3 h after the birth of first piglet. Samples were frozen at -20° C until analysis.
Plasma samples for P4 were analyzed using a commercial radioimmunoassay (RIA) kit (Progesterone RIA kit, MP Biomedicals, CA, USA) and the laboratory analysis of total colostrum IgG was done using commercially available pig IgG ELISA kit (Pig IgG ELISA kit, Bethyl Laboratories, Montgomery, USA).
Results: The average plasma P4 concentration were 3.37 ± 2.06 ng/ml (range 0.93 – 10.71 ng/ml) and 2.62 ± 1.65 ng/ml (range 0.44 – 8.92 ng/ml) (mean ± SD) at the beginning and the end of farrowing respectively. Average IgG concentration (0-3 h) was 74.01 ± 26.77 mg/ml (range 28.57 – 117.80 mg/ml). Sows which had values of P4 > 4.00 ng/ml had a tendency to have lower IgG in colostrum (p = 0.10).
Conclusion: We found that sows with an abnormal P4 concentration at farrowing tended to have lower IgG content in colostrum, which might indicate hormonal disruption during the farrowing, with potential negative effects on piglets IgG uptake.

Disclosure of Interest: None Declared

Keywords: Colostrum, Progesterone, Sow

Residents' ECPHM Session

PO-PW1-260

Effect of oral cobalamin supplementation on fecal Lawsonia intracellularis genome fragments in vaccinated and non-vaccinated weaned pigs.

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Introduction: Porcine proliferative enteropathy (PPE), also known as ileitis, is caused by the obligate intracellular bacterium *Lawsonia intracellularis*. Pigs with subclinical PPE have no specific clinical signs but their production performance is decreased during the growth and fattening period due to a reduced nutrient absorption by immature enterocytes in the small intestine. The majority of cobalamin (vitamin B_{12}), which plays an important role in amino acid metabolism and nucleic acid synthesis, is absorbed at the ileum. Therefore, we evaluated if oral supplementation of cobalamin affects the performance in weaned pigs that are either vaccinated or not against *Lawsonia intracellularis*.
Materials and Methods: Pigs from a selected farm with a confirmed *Lawsonia intracellularis*-infection were included in the study. Twelve pigs each were randomly assigned to 5 different groups (vaccinated [vacc.]/non-supplemented [non-suppl.], vacc./suppl., non-vacc./non-suppl., non-vacc./suppl., and tylosin [non-vacc./non-suppl.]). Corresponding pigs were administered an avirulent live *Lawsonia intracellularis* vaccine, received tylosin (4.5 mg/kg body weight) for the whole study period or cobalamin orally (0.2 mg/kg feed) from day 8 to 21. Fecal samples were obtained from all pigs on day 0, 7, 14 and 21. *Lawsonia intracellularis* was tested using a quantitative fecal PCR test. An ANOVA or MANOVA model was used to compare the average daily weight gain (ADWG), oral supplementation of cobalamin and amount of fecal *Lawsonia intracellularis* genome fragments among the 5 groups of pigs.
Results: Baseline body weights differed significantly among the 5 groups of pigs (p=0.017), caused by lower weight of the tylosin compared to vacc./non-suppl. pigs. The ADWG differed significantly among the groups (p=0.006), with the lowest ADWG observed in the non-vacc./non-suppl. group compared to the remaining 4 groups with a statistical significance of p<0.05 for all except for the non-vacc./suppl. pigs. Levels of fecal *Lawsonia intracellularis* genome fragments differed significantly between the non-vacc./non.suppl. (highest), vacc./non-suppl. (increased during the trial) and tylosin (lowest) group (p<0.001). Genome fragments of fecal *Lawsonia intracellularis* increased faster in vacc./suppl. pigs compared to vacc./non-suppl. pigs (p=0.001) but no difference between non-vaccinated groups (p>0.05).
Conclusion: Cobalamin supplementation over 2 weeks affected fecal *Lawsonia intracellularis* genome fragments in vaccinated but not in non-vaccinated groups of weaned pigs. Whether supplementation of cobalamin over a longer time-period affects the production performance in non-vaccinated weaned pigs warrants further investigation.

Disclosure of Interest: N. Grützner Conflict with: Spezko - Research Fellowship, A. Luehrs: None Declared, E. grosse Beilage: None Declared, H. Nathues: None Declared

Keywords: Lawsonia intracellularis, Vitamin B12, weaning pigs

Residents' ECPHM Session

PO-PW1-263

MRI as an in vivo diagnostic tool for neurological disorders in pigs

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Introduction: A ±7 weeks old male pig was brought to the university clinic for teaching purposes. The piglet was anorexic, growth retarded and soporific. It had an uncoordinated gait, showed intermittent circling to the left, slight tremors over its body and often used its carpi for support. During the locomotion and neurological exams, an unclassifiable ataxia was observed as well as delayed tactile and optic placement reflexes. The neurolocalization was likely the central nervous system (CNS) and the differential diagnosis included traumatic, congenital, infectious, metabolic or toxic causes. In these cases, post-mortem examination is usually the only diagnostic tool used. Diagnostic imaging techniques such as Magnetic Resonance Imaging (MRI) could be of help for an *in vivo* diagnosis in these cases. The aim of this case report is to compare MRI findings with the gross and histologic pathomorfologic changes and to discuss the utility of MRI for *in vivo* diagnosis of CNS disorders in pigs.
Materials and Methods: MRI examination of the neurocranium and cervical spine was performed after administration of gadoterate meglumine (Dotarem®) as a contrast agent with a 1.5 Tesla scanner. Sequences acquired for imaging were: transverse T1-weighted (W) turbo spin echo (TSE), T2-W TSE, T1-W gradient echo (GRE), fluid attenuated inversion recovery and T2* GRE (neurocranium), and T1- and T2-W TSE in transverse and sagittal planes (cervical spine). Subsequently, macroscopic and microscopic post mortem examination was performed.
Results: Multifocal, symmetric, T2-W hyperintense and T1-W iso to hypointense non-contrast enhancing areas were seen in the cerebrum, thalamus, brain stem and cerebellum, compatible with a toxic or metabolic disorder. A diffuse and ill-defined T2-W intramedullary hyperintensity was seen within the dorsal half of the spinal cord from C1 until C6, compatible with oedema or myelitis. Although no macroscopic abnormalities of the neurocranium or cervical spine were found, histology showed a multifocal acute fibrinonecrotizing vasculitis with moderate perivascular oedema and malacia within the brain stem, mid brain, thalamus, hippocampus and basal ganglia, compatible with oedema disease due to Shiga-like toxin produced by *E. coli*.
Conclusion: *In vivo* diagnostic imaging, such as MRI, is useful to narrow the diagnosis of non-inflammatory CNS disorders. In our study, the MRI results were compatible with a toxic or metabolic disorder, but due to the lack of references of MRI studies on pigs, histology was necessary to get a final diagnosis.
Acknowledgement: Katja Bleeker for her help in this case.

Disclosure of Interest: None Declared

Keywords: CNS disorders, MRI, Pigs

Residents' ECPHM Session

PO-PW1-262

Implementing drinking water feed additives strategies in post-weaning piglets: Case Study.

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Introduction: Weaned piglets suffer many stressors such as sudden change of feed, change of pigs groups and passive protection decrease with ageing. The aim of this study was to investigate the effect of the inclusion of a free and buffered organic acids blend (OAs) to drinking water (DW) of weaned pigs on performance in presence or absence of medication to control enteric disease.
Materials and Methods: One-hundred and forty (140) pigs in a conventional herd were allocated after weaning to one of three treatments and monitored during 4 weeks: group (1) Control full medication (FM) (*Amoxycillin trihydrate* (AT) 870 mg/g – 0.46 kg/t (400 ppm) & *Neomycin sulfate* (NS) 600 mg/g – 0.5 kg/t (300 ppm) in feed), group (2) OAs blend + FM (2 L/1,000 L OAs DW + AT&NS), group (3) OAs + reduced medication (OAs DW + AT). Pigs were weighed individually on a weekly basis to determine average daily gain (ADG). Feed intake (ADFI) and water consumption (WC) was recorded at group level.
Results: Average daily gain (g/day) in the different groups were at week 4; (1) 0.228a, (2) 0.238a, (3) 0.265b ($p<0.001$). ADG of the piglets from (3) was significantly higher compared to (1) over 0-2 weeks of the study. Over the 0-4 weeks period (3) treatment was higher compared to control and (2).
The Feed conversion rates (FCR) in the different groups were at week 4; (1) 1.76a, (2) 1.70a, (3) 1.46b ($P<0.001$). Thus 0,3 better in (3) than (1).
The Water Consumptions (L/pen) were at week 3 (1) 11.4a, (2) 24.5b, (3) 23.8b ($P=0,004$), and at week 4, (1) 13.9a, (2) 31.6, (3) 28.4b ($p=0,014$). Pigs receiving OAs in DW had significantly higher water usage than group (1) in weeks 3 and 4.
The pigs from group (3) tended to grow faster during each week, but the difference was only significant during week 4 ($P<0.001$). There was no significant difference in ADFI between treatments. FCR for (3) program improved by 1.0 compared to (1) and (2) in week 1 ($P<0.05$), while in week 2 and 3 no significant differences were found. In the fourth week of the study FCR was improved by 0.3 in (3) compared to group (1) ($P=0.001$). The (3) resulted in significantly better FCR than (1) across the first two weeks of the experiment, and significantly better than (1) and (2) during weeks three and four on accumulative basis.
Conclusion: The group with a blend of free and buffered organic acids (OAs) together with a reduced medication (group 3) had a better ADG and FCR during the nursery period. The better performance may result from the positive effect of OAs on microbial balance in the intestines.

Disclosure of Interest: None Declared

Keywords: Drinking additive, Performance, piglets

Poster Abstracts

Vaccinology & Immunology

PO-PC03-001

BEHAVIOUR IN PIGLETS VACCINATED WITH TWO DIFFERENT VACCINES BASED ON DIFFERENT ADJUVANTS

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Introduction: Every day, millions of piglet vaccinations are carried out to protect the piglets against infectious diseases. Due to side effects, some vaccinations might actually by themselves provide a challenge to the piglets´ wellbeing, especially the vaccines that have an oily adjuvant. The present study examines the impact of two different vaccines against PCV2 and M hyo on the behavior of piglets after vaccination.
Materials and Methods: At 4 weeks of age, piglets from a commercial pig herd were weaned and transported to a sectionized nursery and distributed in pens according to size. Piglets of the same size were sharing one feeder.
The day after weaning, all piglets were vaccinated against PCV2 and M hyo. Piglets on the right side of each feeder were vaccinated with vaccine A with an aquous polymer as adjuvant (FLEXcombo®, Boehringer Ingelheim), and piglets on the left side of the feeders were vaccinated with vaccine B with an oil-in-water adjuvant (Porcilis PCV M Hyo, MSD Animal Health). Vaccinations were done according to label, including warming of vaccine B before injection. 2x2 pens around 2 feeders were recorded on video cameras after vaccination (Garmin VIB HD action camera), with 83 pigs/group in total.30 minutes after vaccination, an approachability test was made, counting the number of pigs approaching a human observer. The result of this test was compared to a baseline approachability test made on the same time on the day of weaning. From the video recordings, the number of piglets visiting the feeder and drinker was counted. The activity level of the piglets was summed up as number of piglets not lying down with a 5 minutes interval. Statistical analysis was made with Fishers Exact test, with p=0.05 as level of significance.
Results: Pigs vaccinated with vaccine B were significantly less willing to approach a human observer in the pen after vaccination than pigs vaccinated with vaccine A (p<0,001). Before vaccination there was no difference in the willingness to approach (p=0.534). Vaccination with vaccine B resulted in 48% reduction of the number of visits to the feeder and 73% reduction in the number of visits to the drinking nipple compared to vaccine A. The overall activity level was significantly different (p<0.001/0.03), with pigs vaccinated with vaccine A being more active.
Conclusion: This study shows that the adjuvant has a significant impact on the behavior of piglets after vaccination. Vaccinations with an oily adjuvant lead to a reduced level of activity and reduced intake of feed and water. This might give the piglets a drawback in growth rate compared to piglets vaccinated with a milder adjuvant.

Disclosure of Interest: B. Grosse Liesner Conflict with: Boehringer Ingelheim

Keywords: behaviour, Vaccination PCV2-Mycoplasma

Vaccinology & Immunology

PO-PC03-003

Circumvent® PCV M G2: PCV2d Challenge Study

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Introduction: Porcine circovirus type 2 (PCV2) remains an economically important disease, at least in part due to the high rate of disease in pigs that are not successfully immunized for various reasons. One potential cause for reduction in vaccine efficacy is the emergence of new PCV2 variants including one variant that was first reported in China and has apparently spread to the US where it now appears to be the dominant variant in pigs with clinical PCV2 infection. The objective of this study was to evaluate the efficacy of Circumvent® PCV M G2 (PCVM-G2) against a mutant PCV2d infection under laboratory conditions with a previously used PRRSV co-infection challenge model.
Materials and Methods: Pigs were tagged, weighed, their gender recorded, allotted to treatment group within litter and vaccinated at 2-5 days of age (DOA). Four treatment groups were evaluated: Group A- non-vaccinated controls; Group B- vaccinated twice with 1 mL at 3 DOA & 3 weeks of age (WOA); Group C- once, 2mL, 3 WOA; and Group D- twice, 1 mL, 3 & 6 WOA. After movement to an isolation facility, the pigs were bled at approximately 3, 6, 9, 11, 12 and 13 WOA. The pigs were challenged at 9 WOA, the inoculum contained a PCV2d tissue homogenate and PRRSV, and was administered intra-nasally. Necropsies were performed at 13 weeks of age and fresh and fixed lymphoid tissues were collected. Quantitative PCR, IHC, histopath and IFA were done using standard methods.
Results: PCR and sequencing revealed that PCV2a infection was occurring prior to challenge, eliminating 4 of 12 litters. The IFA titers (no. pigs) at necropsy by group were: A- <20 (n=8); B- 1754 (n=11); C- 2079 (n=10); and D- 4777 (n=10). The average copies per reaction in serum (11, 12 and 13 WOA combined) and tissue (composite of 4) for the control group was 5.01 X 10^{10} and 1.52 X 10^{10}, respectively. The percent reduction in copies per reaction in serum and tissues for the vaccinated groups was: B- 98.4% and 99.9%; C- 99.9% and 100.0%; and D- 100.0% and 100.0%. Microscopic lesion and IHC scores were significantly lower in the vaccinated groups compared to the control group. For all parameters, the vaccinated groups were similar to each other.
Conclusion: Overall, PCVM-G2 was able to protect pigs against a PCV2d challenge. The percent reduction in viremia and tissue infection levels compared to controls ranged from 98.4% to >99.9%. The level of viremia and tissue infection was higher in control pigs compared to previous studies, suggesting that PCV2d may be more virulent than the previous PCV2b variant used in this challenge model.

Disclosure of Interest: B. Thacker Conflict with: Merck Animal Health, R. Schlueter Conflict with: Merck Animal Health, D. Madson Conflict with: Merck Animal Health, R. Hesse Conflict with: Merck Animal Health, Conflict with: Merck Animal Health

Keywords: PCV2 vaccine, PCV2d

Vaccinology & Immunology

PO-PC03-004

Outbreak management in a Naïve farm infected with PRRSV RFLP 1-7-4

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Introduction: D. Linhares et al. Prev Vet Med 116, 2014 described Time to Stability (TTS) as time in weeks it took to produce PRRSV negative pigs at weaning, and Time to Baseline Production (TTBP) to represent time to recover to the number of pigs weaned prior to PRRSV detection. In Linhares' study, TTS was achieved in 26.4 wks (25th quartile: 21.6 & 75th quartile: 33) and TTBP in 21 wks (25th quartile: 16 & 75th quartile: 24) in farms infected with PRRSV 1-4-4. These are important metrics to help to evaluate PRRS control & elimination strategies. The objective of this field case study was to describe a 1-7-4 PRRSV outbreak management and its outcome in a Naïve breeding herd.
Materials and Methods: The analysis was conducted in a 2600 naïve sow farm that broke with PRRSV RFLP 1-7-4 in April 21st 2015. A measurement system for severity, duration and recovery of a reproductive PRRS outbreak was set using Statistical Process Control (SPC). Parameters analyzed in SPC-Shewhart charts: Sow mortality; Farrowing rate; Avg born alive/ birth litter and PWM % identifying signals out of control, also Weaned pigs / Litter was analyzed using SPC-EWMA chart to determine TTBP. The production records were imported from PigWIN to MINITAB and the period of evaluation was from week #1-2014 to week #40-2015. For TTBP baseline we took the previous 28 weeks before the outbreak.
In addition, PCR PRRS in 30 piglets was performed to add Time to Stability (TTS) to the analysis.
Interventions are summarized as homogenization of the breeding herd with vaccination with unidirectional flow in replacement gilts. They were implemented immediately after PRRSV was diagnosed.

Replacement gilts:
Stopped additions of new groups in gilt development unit (GDU)
Place Beta-gro into gilts diet
Fostera® PRRS to all gilts (June 1st)
Exposure to all gilts via weaned pigs in GDU (June 15th)
Fostera® PRRS to all gilts (June 29th)

Breeding herd blanket vaccination:
1st vaccination: May 1st 2015
2nd vaccination: June 2nd 2015
3rd vaccination: September 9th 2015
Modified McREBEL
Results: This case study described metrics outcome from interventions to mitigate a PRRS 1-7-4 reproductive outbreak in a Naïve breeding herd
Time to Stability was reached 26 weeks after interventions implemented
Outbreak severity measure as SPC out of control signals
Sow mortality: 6 wks
Farrowing rate: 21 wks
Born alive / birth litter: 19 wks
PWM: 9 wks
Time to Baseline Production was reached 22 weeks after intervention implemented

Conclusion: PRRS interventions based on reducing circulation and managing whole herd immunity (gilts and breeding herd vaccination with Fostera® PRRS) helped to minimize and managed PRRSV 1-7-4 impact in a naïve population

Disclosure of Interest: None Declared

Keywords: Outbreak Management, PRRS RFLP 1-7-4

Poster Abstracts

Vaccinology & Immunology

PO-PC03-010

Development of an elongated bacterial vaccine against Erysipelothrix rhusiopathiae infection

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Introduction: Phagocytosis of microbes by monocytes is a crticial factor to enhance vaccine effects. It was previously reported that the bigger size of bacteria resulted in higher probability of phagocytosis compared with the normal ones. For instance, *S.pneumoniae* with longer chains were easily uptaken *in vitro* due to the deposition of complements. However, it is not well proved whether the uptake of the bigger size of bacteria induces acquired immunity *in vivo* and enhances vaccine effects. Here, we proposed the system to elongate the bacteria, *E.rhusiopathiae* (ER), and applied this system to examine the vaccine effects of elongated bacteria by *in vivo* administration. Our results indicate that elongation of bacteria is required for enhancing the vaccine effect but not sufficient.
Materials and Methods: ER was grown with cephalexin, inactivated by formalin, and supplemented with micro emulsion as adjuvant. Mice were immunized subcutaneously with the vaccines. For the detection of anti-SpaA (a photogenic factor of ER) antibodies by ELISA, blood samples were collected. After the immunization, the mice were challenged with ER.
Results: In 3 h incubation with cephalexin, the length and number of elongated bacteria was 3 and 0.1 times larger than those of the non-treated bacteria, respectively. When they were administrated to mice, the survival rate in a group of mice vaccinated with elongated bacteria (*A*), non-treated bacteria (*B*) and sole cephalexin mixed with non-treated bacteria (*C*) are 80%, 20% and 10%, respectively. Titer of anti-SpaA antibody in group *A* showed more than twice larger than that of group *B and C*. These results indicated that vaccination with elongated bacteria potentiate to induce stronger protective immunity against ER infection than that with control bacteria.
To examine the vaccine effects of components within elongated bacteria, bacteria after incubation with or without cephalexin were collected. When bacteria vaccine without supernatants were administrated to mice, there was no difference in both the survival rate and titer of anti-SpaA antibody between group *A* and *B*. These results suggest that not only intra-cellular components within elongated bacteria but also the secretion suspended in the supernatants contributes to the enhancement of vaccine effects.
Conclusion: Vaccination with elongated bacteria including the supernatants induces higher immunological response than one with non-treated bacteria, resulting in protection of ER infection. This effect does not arise from the cell components located inside the cells, rather indicating that elongation of bacteria by cephalexin induces the secretion of some yet-uncharacterized bacterial components to enhance vaccine effects.

Disclosure of Interest: None Declared

Keywords: elongated bacteria, Erysipelothrix rhusiopathiae, vaccine efficacy

Vaccinology & Immunology

PO-PC03-015

Effect of ileitis oral vaccination against Lawsonia intracellularis on antibiotic use reduction and performance improvement in a Spanish company

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Introduction: *Lawsonia intracellularis* (L.i.) is the causative agent of porcine proliferative enteropathy (PPE). PPE is a relevant economic enteric disease that causes diarrhea and reduces weight gain in growing pigs (1). The subclinical form produces as well a negative impact on performance and farm economics. L.i is endemic in most of the Spanish farms (2). The aim of this study was to evaluate the efficacy of Enterisol® Ileitis (Boehringer Ingelheim Vetmedica GmbH) in a Spanish commercial company.
Materials and Methods: This study was conducted in a 1200 sows farrow to finish farm located in the eastern region of Spain. Pigs at fattening were suffering subclinical ileitis and L.i. infection was confirmed by ELISA (IgG). A total of 12.120 fattening pigs were included in the study (6611 non-vaccinated and 5509 vaccinated with the oral nonvirulent live vaccine Enterisol® Ileitis (Boehringer Ingelheim Vetmedica GmbH). Thus, 10 weekly batches were vaccinated and 12 alternate's batches were kept unvaccinated in order to minimize the seasonal impact on results. The piglets were orally vaccinated via drinking water at weaning in the nursery unit using Thiosulfate Blue (Boehringer Ingelheim Vetmedica GmbH) as stabilizer. All the animals were raised under similar conditions. The parameters recorded were: average daily gain (ADG, kg/d), feed conversion rate (FCR), FCR corrected (FCRc.), mortality rate (%) and antibiotics costs (€). Data has been analysed using ANOVA with SPSS v.15.0 (SPSS Inc., Chicago, IL, USA) software.
Results: The results are summarized in Table 1. Due to differences on initial and final weight, corrected FCRc. (18-100) was used.The reduction on antibiotic use in vaccinated group represents 23,6% compared to those animals that were not vaccinated.The average mortality was 7,5% lower in vaccinated group (4,57 % vs 4,94 %). In addition corrected FCRc was 57 g less and ADG was 10 g/day better also in vaccinated group. Statistical differences could have been obtained in these performance data with a higher sample size (n).
Conclusion: In this field experience, it was demonstrated that antibiotic use was significantly reduced by the vaccination with Enterisol Ileitis®. This fact means that the immunization improved pig´s health. Then growing parameters and mortality were numerically better to.

Disclosure of Interest: None Declared

Keywords: Antiobiotic reduction, Enterisol ileitis, performance

Vaccinology & Immunology

PO-PF3-023

Evaluation of diagnostic tests for Circumvent PCVM G2 compliance monitoring

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Introduction: Effective immunization against porcine circovirus type 2 (PCV2) and Mycoplasma hyopneumoniae (Mhp) is dependent on strict compliance with vaccination protocols. Circumvent® PCVM G2 (PCVM-G2) is a poly-antigen vaccine composed of PCV2, Mhp and baculovirus antigenic components and results in development of measurable antibody responses after vaccination. Accordingly, serodiagnostic tools are potentially useful for evaluating vaccination compliance. The objective of this study was to evaluate several serological assays for potential use for PCVM G2 compliance monitoring. The poly-antigenic nature of the vaccine allows for the possibility of multiple serological evaluations of compliance whether the vaccine is administered by label with either one or two dose regimens.
Materials and Methods: Three week old pigs were allotted to the following PCVM-G2 vaccinated groups: Group A pigs (n=30) were vaccinated with a single 2 ml dose at 3 weeks of age (WOA); Group B pigs (n=30) were vaccinated with a 1 ml dose at 3 and 6 WOA; Group C (n=15) pigs served as non-vaccinated controls; and Group D pigs (n=30) were vaccinated with 1 ml at 3 and 6 WOA by the operation's vaccination crew. Pigs were bled at three and nine weeks of age. Serum samples were evaluated for PCV2 antibodies by full range dilution immunofluorescence assay (IFA), four dilution IFA, Ingenasa PCV2 IgG ELISA and ISU-VDL PCV ELISA; Mhp antibodies by the IDEXX ELISA; and baculovius antibodies by the ISU-VDL BV ELISA.
Results: The results provide the following findings: 1) consistent PCV2 immune response was observed at 9 WOA for groups A, B and D with the full range dilution IFA and the Ingenasa ELISA; 2) consistent PCV2 immune response was observed in groups B and D with the four dilution IFA; 3) consistent Mhp immune response was observed in groups B and D with the IDEXX ELISA; and 4) consistent baculovirus immune response was observed with the BV ELISA in group B only. Group C pigs remained consistently negative on all assays at 9 weeks of age.
Conclusion: All of the assays produced a consistent immune response when the vaccine was used in a two dose regimen. With the one dose regimen, only PCV2 antibodies were measurable by the full range dilution IFA and the Ingenasa ELISA. In the field, positive results with all of the PCV2 and Mhp assays could be due to natural infection so interpretation of the results needs to be considered in light of potential field exposure prior to or during the vaccination period. However, this study provides guidance for interpreting negative results. Accordingly, these assays can be used as indicators of compliance failure.

Disclosure of Interest: B. Thacker Conflict with: Merck Animal Health, T. Girard: None Declared, P. Thomas: None Declared, J. Creel Conflict with: Merck Animal Health

Keywords: Mycoplasma hyopneumoniae, PCV2 vaccine, Serology

Vaccinology & Immunology

PO-PF3-041

Field efficacy of two PCV2 and Mycoplasma hyopneumoniae vaccine combinations under Brazilian field conditions

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Introduction: The objective of this study was to investigate the efficacy of two combinations of vaccines against PCV2 and *Mycoplasma hyopneumoniae (M hyo)* under Brazilian field conditions.
Materials and Methods: This study was performed in a farrow-to-finish farm located in Minas-Gerais province, with a history of clinical PCVD and enzootic pneumonia during growing/finishing, and lack of homogeneity at slaughter time. A group of 896 healthy 3-week-old piglets was included in the study and allocated to the 4 treatment groups, using randomization according to sex and initial live weight. Pigs were administered CIRCOVAC®, Merial (0.5 mL) and SPRINTVAC®, Merial (2.0 mL) in groups T1 and T3 (VAC groups). Another PCV2 vaccine (1.0 mL) and another *M hyo* vaccine (1.0 mL) combination was tested in groups T2 and T4 (X groups). Vaccines were administered at weaning at the same time but separately in T1 and T2 and mixed prior to administration in T3 and T4. were housed in 7 pens of about 35 pigs until the end of the study. Individual bodyweights at 24, 64 and 148 days of age were recorded. Mortality and culling as well as pen feed consumption were assessed for the nursery period (24-64 days of age) and the fattening period (64-148 days of age). Coughing was recorded. Lungs in 80 pigs per group were inspected using Madec's grid. Serology was performed in 10 pigs per group to assess PCV2 and *M hyo* circulation.
Results: PCV2 and *M hyo* natural circulation were confirmed serologically. On average, weaning-to-slaughter (W-S) average daily weight gain (ADWG) in VAC groups was +17 g/day ($p<0.05$) higher than in X groups. W-S ADWG was 827, 826 and 817,803 g/day in groups T1, T3 and T2, T4 respectively with a statistically better growth in T1 and T3 as compared to T4 ($p<0.05$). Slaughter weights at 148 days in groups T1 and T3 were +3.2 and +3.0 kg higher than weights in group T4 ($p<0.05$). Pigs in group T2 were 1.2 kg lighter than pigs in group T1 ($p>0.05$). No difference in feed conversion ratio was observed. Throughout the study, mortality and culling rate was lower ($p<0.05$): 5.4%, 6.7% vs 5.4%, 13.8% in groups T1, T3 vs T2, T4, respectively. Coughing index was significantly lower in T3 (1.1) as compared to X groups (T2: 1.94 and T4: 2.26) but not compared to T1 (1.42). At the abattoir, magnitude of the lesions was low whatever the group and no difference in prevalence (84 to 86%) was observed between VAC and X groups.
Conclusion: Under the conditions of the study, combining CIRCOVAC and SPRINTVAC either mixed prior to administration or injected separately provided comparable field protection against PCV2 and *M hyo.* In addition, mixing these very same products provided better protection than mixing the other combined products tested.

Disclosure of Interest: R. W. Pinheiro: None Declared, E. Bordin: None Declared, O. Merdy Conflict with: Merial S.A.S., F. Joisel Conflict with: Merial S.A.S.

Keywords: combination vaccine, Mycoplasma hyopneumoniae, PCV2

Poster Abstracts

Vaccinology & Immunology

PO-PF3-044

Water in oil adjuvant selection for the formulation of one-shot safe bacterial vaccines for swine

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Introduction: Oil adjuvants are extensively used in swine inactivated vaccines. Classical oil based swine vaccines consists in oil-in-water emulsions, or water-in-oil-in-water double emulsions. These adjuvants induce a strong short term response, but can induce hyperthermia and more rarely anaphylactoid shocks after injection, especially with reactive antigens such as *Actinobacillus pleuropneumoniae* (APP). The use of water-in-oil adjuvants is an option to reduce the risks of short term reactions after injection. Water in oil vaccines for swine must however be optimized carefully to avoid risks of lesions at the injection site at slaughter. Here we show that the selection of an adapted water-in-oil adjuvant allows the formulation of a safe APP vaccine with a reduced antigenic load compared to oil-in-water vaccine.
Materials and Methods: APP S2 vaccines were formulated with 2 different water-in-oil adjuvants (WO1 and WO2). 3 vaccines were formulated for each adjuvant with a range of APP concentration: 100% (2.25 10^8 CFU/ml), 10%, 1%. 80 6-week old pigs were randomly separated in 7 test groups and 1 non-adjuvanted control group. At D0, 3 groups received 1 ml of WO1 based vaccines at 100%, 10% and 1%, 3 groups received 1 ml of WO2 based vaccines at 100%, 10% and 1%, 1 group received 2ml of oil in water (OW, Montanide™ ISA 15A VG) classical vaccine at 100% of antigen concentration. Body temperature and local reactions were measured in all groups at 4 and 24h after injection, and carcass quality was also assessed at slaughter at D120. Blood samples were taken at D0, 28, 80 and 120, and specific IgG1 and IgG2 antibody titers against APP were measured by ELISA titration.
Results: Water in oil vaccines did not induce any body temperature increase, local or general reaction at 4 and 24h after injection, whereas OW vaccine induced a 1°C increase in temperature at 4h after injection. With 100% antigenic load, both WO1 and WO2 induced significantly higher humoral responses to APP than classical OW vaccine, but also induced non acceptable local reactions at slaughter. With 10% of antigenic load, 1 ml injection of WO2 based vaccine induced a humoral response similar to the injection of 2ml of OW based vaccine containing 100% antigenic load, but did not induce injection site lesions at slaughter.
Conclusion: These results show that water in oil adjuvants can allow the formulation of safe bacterial swine vaccines. However, the selection of an adapted adjuvant and antigenic load and vaccine dose adjustment is critical to avoid lesions at slaughter. Water in oil adjuvants can allow the formulation of one-shot vaccines for pigs with limited amount of antigen, with low pyrogenicity and low risks of anaphylactoid shocks.

Disclosure of Interest: J. Ben Arous Conflict with: SEPPIC, N. Versille Conflict with: SEPPIC, L. Dupuis Conflict with: SEPPIC, H. Imbault: None Declared

Keywords: APP, One-shot vaccine, Vaccine adjuvant

Vaccinology & Immunology

PO-PF3-045

Efficacy of ERYSENG® PARVO LEPTO in swine farms with leptospirosis problems

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Introduction: The aim of this study was to assess the efficacy of the ERYSENG® PARVO LEPTO vaccine on swine farms with reproductive problems due to leptospirosis. The efficacy outcomes were compared with a negative control group.
Materials and Methods: A controlled, blinded field study was performed in 116 nulliparous gilts from 3 different commercial farms in Brazil. The farms had reported leptospirosis problems and the animals were positive to Leptospira at the beginning of the trial. Animals were randomly assigned to an ERYSENG® PARVO LEPTO group (n=73) and a negative control group (n=41). According to the recommended vaccination schedule, the first dose was administered between 8 and 6 weeks before insemination and the second dose was applied 21 days later. Animals from the control group received PBS following the same schedule as the vaccinated group. Reproductive parameters from all animals included in the trial were registered and stillborn, mummies and abortions were collected and analysed to detect Leptospira by PCR. Statistical analyses were undertaken using the Mann-Whitney test ($p<0.05$) for reproductive parameters and the Chi-square test for Leptospira PCR ($p<0.05$).
Results: Following the use of ERYSENG® PARVO LEPTO on the farm, the percentage of abortions was reduced from 5% in the control animals to 0% in the vaccinated group. Moreover, there were statistical differences between the control animals and the vaccinated ones in the number of stillborn/litter (1.02 versus 0.47, respectively) and mummies/litter (0.44 versus 0.12, respectively). The stillborn, mummies and abortions were submitted to the laboratory (University of São Paulo) and analysed to detect Leptospira by PCR. ERYSENG® PARVO LEPTO significantly reduced the number of positive PCR samples for Leptospira compared to the control animals (57% positive in the control animals versus 14% in the vaccinated ones).
Conclusion: Vaccination with ERYSENG® PARVO LEPTO was shown to be efficacious against Leptospira infections by a reduction in the percentage of abortions, a reduction in the number of stillborn and mummies per litter and a reduction in Leptospira infections in the vaccinated animals.

Disclosure of Interest: C. F. C. Scherer Conflict with: Hipra, A. Puig Conflict with: Hipra, A. Camprodon Conflict with: Hipra, M. Coll Conflict with: Hipra, M. Cesio Conflict with: Hipra, R. March Conflict with: Hipra

Keywords: Leptospira sp, reproductive parameters, vaccine efficacy

Vaccinology & Immunology

PO-PF3-051

Experimental and field evaluation of the performance of two ELISA methods to detect seroconversion against swine erysipelas in pigs.

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Introduction: Swine erysipelas (SE) is one of the diseases of greatest prevalence and economic importance in pork production. A protective role of specific antibodies against *Erysipelothrix rhusiopathiae*, the causative agent of SE, has long been suggested. Mass vaccination against SE is the most commonly used approach to control SE, which relays heavily on the development of a strong herd immunity. Despite the efficacy of existing vaccines, inadequate vaccination protocols may lead to the emergence of SE outbreaks even in vaccinated herds. Serological monitoring is, therefore, of paramount importance in disease control. The aim of this study was to compare two commercially available ELISA kits regarding their competence to assess immunization of SE vaccinated and naturally infected pigs.
Materials and Methods: A total of 458 sera of known SE status were tested in duplicate for anti-SE antibodies by the indirect ELISA 1 (Civtest Suis SE/MR, Hipra, Spain) and 2 (Ingezim Mal Rojo 1.1.MR.K1, Ingenasa, Spain), as per manufacturer's instructions. Samples were divided into three groups: G1 with 111 SE-negative sera (certified in origin), G2 with 122 SE-positive sera after infection (87 coming from vaccination-challenge studies and 35 from natural infection), and G3 with 225 SE-positive sera collected at time intervals after vaccination (5 commercially available SE vaccines) under experimental conditions (not challenged).
The results were assessed for sensitivity (Se), specificity (Sp), agreement and correlation (Rho) among the assays using *kappa* statistic and Spearman Rank Order Correlation. Statistical analyses were performed using MedCalc and SPSS software.
Results: The results of G1 and G2 were similar in both ELISAs, which in turn showed high sensitivities and specificities, although the ELISA 1 performed marginally better than ELISA 2 in terms of Se (100% and 97.5%, respectively) and Sp (100% and 98.7%, respectively). In addition, an almost complete agreement (kappa=0.96), and a strong positive correlation (Rho=0.86) were found between the results from both tests using samples in G1 and G2. Comparison of the results obtained with the two kits using samples in G3, showed a substantial agreement (kappa=0.73) and a strong positive correlation (Rho=0.86).
Conclusion: These results demonstrate that ELISAs 1 and 2 are suitable for the evaluation of antibody response in SE infected and vaccinated pigs, with good performance characteristics. Also, these kits seem to be useful to assess the immunization of pigs after vaccination with different commercially available vaccines and immune status of pig populations.

Disclosure of Interest: None Declared

Keywords: Diagnostic, Erysipelothrix rhusiopathiae, serology

Vaccinology & Immunology

PO-PF3-062

Field trials evaluating the efficacy of porcine epidemic diarrhea vaccine (Harrisvaccine) in the Philippines

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Introduction: Porcine epidemic diarrhea (PED) is an economically devastating enteric disease characterized by vomit and diarrhea. Currentcontrol method including oral administration with PED infected intestine has been used with some degree of success. However, this management protocol provide potential risk of pathogen recontamination into the herd. *Porcine Epidemic Diarrhea Vaccine, RNA (*Harrisvaccines, IA, USA) is the first USDA conditionally licensed PEDV vaccine. Therefore, the study was conducted to evaluate the efficacy of the PEDV vaccine in the induction of antibody in colostrum and milk samples.
Materials and Methods: The study was conducted in two sow herds (herds A and B) located in the Philippines. In each herd, 50 sows were randomly selected based on the stratification of parity into 2 treatment groups including Control and Trial. Sows in Control group were left with no vaccination and sows in Trial group were intramuscularly injected once with PEDV vaccine (*Porcine Epidemic Diarrhea Vaccine, RNA (*Harrisvaccines, IA, USA) at 7 days prior to farrow. Blood samples were collected from sows at -7, 0, 7 and 14 days post farrow (DPF). Colostrum was collected within 3 hours after labor and milk samples were collected at 7, 14 and 21 DPF. Two piglets from each litter were randomly selected and ear-tagged and blood samples were collected at 14 and 21 days of age. Serum, colostrum and milk samples were assayed for the presence of PEDV specific antibody by viral neutralization (VN) assay, and ELISA IgA and IgG, specific to spike protein.
Results: The results from 2 herds demonstrated that sows vaccinated with *Porcine Epidemic Diarrhea Vaccine, RNA (*Harrisvaccine, IA, USA) had significantly higher VN titer, ELISA IgA and ELISA IgG compared to the non-vaccinated sows. PED outbreak was occurred in one herd following the implementation of vaccine. The effects of the outbreak including the number of pigs weaned and pre-weaning mortality was determined. The results demonstrated that sows vaccinated with PEDV vaccine weaned significantly higher number of weaned pigs, although slight clinical diarrhea was observed. This suggested the protective efficacy provided by the vaccine.
Conclusion: The results show that sows intramuscularly injected with *Porcine Epidemic Diarrhea Vaccine, RNA* had significantly higher antibody as determined by VN titer, ELISA IgG and IgA in colostrum and milk samples compared to non-vaccinated sows. In addition, vaccinated sows weaned significantly higher number of weaned pigs following the outbreak.

Disclosure of Interest: None Declared

Keywords: porcine epidemic diarrhea virus; efficacy; vaccine

Poster Abstracts

Vaccinology & Immunology

PO-PF3-063

Effect of supplemental Hostazym® X xylanase complex on immune regulation, gut health and growth performance of nursery pigs.

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Introduction: Non-Starch Polysaccharides (NSP), which are indigestible to monogastric animals, are known to increase intestinal viscosity as well as to interact with gut integrity and immune function. The objective of this trial was to identify an effect of a xylanase complex on gut viscosity, intestinal development and immune status in piglets, and if that response was influenced by a high inclusion of DDGS in the diet.
Materials and Methods: A total of 40 crossbred pigs (10.7 ± 1.2 kg initial BW at 6 wk of age) were used in a 21-d trial to evaluate effects of supplemental xylanase (Hostazym® X, Huvepharma Inc.) in maize-soy-based nursery diets on digesta viscosity, gut health and growth performance. Pigs were individually housed and randomly allotted to 4 dietary treatments in a 2 × 2 factorial arrangement (n=10/treatment, 0 or 1500 EPU/kg xylanase and 0 or 30% DDGS). All animals had free access to water and feed. Body weight and feed consumption were recorded weekly. Plasma samples were collected on d 19 to measure TNF-α (Tumor Necrosis Factor-alpha), being a pro-inflammatory cytokine and important immune mediator. On d 21, all pigs were euthanized to collect tissues from duodenum, jejunum, and colon for TNF-α evaluation and gut morphology. Digesta samples from distal jejunum were collected to measure viscosity. Results were analysed using SAS (SAS Inst. Inc) with significance indicated at $p < 0.05$.
Results: During the entire period, supplementation of the xylanase increased ($p < 0.05$) ADG (616 vs. 660 g/d, resp.) of nursery pigs, whereas DDGS (30%) did not affect ADG (628 vs. 648 g/d, resp.). There was no interaction between the 2 factors, indicating that the effect of xylanase on ADG was independent from the use of DDGS in the feed. Use of DDGS increased ($p < 0.05$) viscosity of jejunal digesta (1.86 vs. 2.38 cP, resp.), whereas the supplemented xylanase reduced ($p < 0.05$) viscosity level (2.27 vs. 1.96 cP, resp.). Plasma TNF-α level was decreased ($p < 0.05$) by the supplementation of xylanase (108.45 vs. 69.87 ng/ml, resp.). Use of DDGS reduced ($p < 0.05$) villus height:crypt depth ratio (1.46 vs. 1.27, resp.) in the duodenum. Supplemental use of the xylanase increased ($p < 0.05$) crypt depth (360 vs. 404 µm, resp.) in the duodenum.
Conclusion: This study indicated that the supplemented xylanase complex improves growth performance and reduces inflammatory status of nursery pigs, by reducing digesta viscosity and plasma TNF-α levels, regardless of the dietary use of DDGS. It was suggested that oligosaccharide end products, from increased NSP hydrolysis through Hostazym® X addition, might have inhibited inflammatory response and enhanced defence.

Disclosure of Interest: None Declared

Keywords: inflammatory response, nursery pigs, xylanase complex

Vaccinology & Immunology

PO-PF3-064

Streptococcus suis autogenous vaccines in French farms :
Benefits of the vaccination of sows on mortality and antibiotic use in their offspring

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Introduction: *Streptococcus suis* (*S. suis*) is a major bacterial pathogen in pigs. Vaccination is a foremost strategy to reduce the impact of the infection and the antibiotic consumption due to this pathology. In France there is no available vaccine and autogenous vaccines are widely used, but there are no accurate and repeatable criteria for evaluating the efficiency of the vaccination. Field clinical observation on several farms is thus a tool for veterinarians to monitor *Streptococcus suis* vaccination.
Materials and Methods: In a French producer group, an evaluation of the vaccination was based on the comparison of the mortality rate during the weaning period, from 3 to 12 weeks of age and the oral amoxicillin and TMP Sulfa consumption from weaning to slaughter, before and after vaccination of the sows. The periods before and after vaccination were variable, depending on the date of implementation of the vaccine, varying from 9 (3 farms) to 12 months (4 farms). No changes in the vaccination program or in the management system were noticed during these periods. Among the 19 farms vaccinated, 7 were able to show reliable data. This represented 1700 sows and 47883 piglets before vaccination and 46144 after vaccination. The vaccination protocol was the same: 2 injections of an oil-adjuvanted autogenous vaccine 6 and 3 weeks before farrowing and then one booster 3 weeks before each farrowing. The symptoms of the disease occurred during the post-weaning period and were caused by *S. suis* serotype 2 for 6 farms and S. suis serotypes 7 and 9 for 1 farm.
Results: Mortality rate during the weaning period was 4.53% before vaccination and 2.25% after, which represented a decrease of 50%. Statistical analysis was performed on GraphPad Prism 6. A Wilcoxon matched-pairs signed rank test was used and showed a p value of 0.0156.
Antibiotic consumption: despite the fact that there was no statistical difference because of a high variation among farms, the total Amoxicillin and TMP Sulfa consumption in g/100 kg from weaning to slaughter decreased by 85%, from 22.637 to 3.39. Among 6 farms which used amoxicillin or TMP Sulfa in feed at weaning, 4 completely stopped and 2 divided by 2 their consumption.
Conclusion: Even if evaluation of the efficiency of a *Streptococcus suis* autogenous vaccine is difficult in the field, good feedback from farmers is the first among criteria to be observed. It was not possible to monitor the average daily weight gain, a potentially excellent tool while waiting for the results of monitoring the protective immune response.

Disclosure of Interest: None Declared

Keywords: autogenous vaccine, Streptococcus suis

Vaccinology & Immunology

PO-PF3-071

Evaluation of Fostera® PRRS cross-protection against a contemporary Linage 1 (RFLP 1-7-4) PRRS virus

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Introduction: The objective of this study was to evaluate the protective efficacy of Fostera® PRRS in pigs vaccinated at three weeks of age and challenged with a virulent Lineage 1 PRRSV (RFLP 1-7-4) at seven weeks of age.
Materials and Methods: The study was conducted in a BSL-2 containment facility at Midwest Veterinary Services Inc. located in Oakland Nebraska. The challenge strain was collected and isolated in 2015 from a farm located in North Carolina that suffered severe reproductive and respiratory clinical signs and showed an ORF5 nucleotide genetic distance of 13.4% from Fostera® PRRS. Forty-six healthy, PRRS-negative pigs were obtained from a commercial sow farm at ~3 weeks of age. Forty pigs were randomly allocated to two treatment groups: T01 Non-vaccinated (N=20) and T02 Vaccinated (N=20). A strict control group (NTX) also was included in the study design as an environmental control that received neither vaccination nor challenge (N=6), NTX pigs were humanely euthanized to monitor for confounding disease factors. Pigs were vaccinated at ~3 weeks of age and comingled four weeks post-vaccination then challenged with virulent PRRS 1-7-4. After challenge, T01 and T02 groups were observed for 10 days and humanely euthanized for lung lesion evaluation
The primary variable was macroscopic lung lesions, while secondary variables included incidence of clinical signs, level and duration of viremia and Average Daily Gain (ADG) post-challenge.
Results: The T01 (Non-vaccinated) group remained PRRSV negative throughout the vaccination period and no confounding disease factors were detected in the strict control group (NTX).
T02 (vaccinated) had significantly fewer lung lesions (P=0.0066) as compared to the T01 (non-vaccinated) group. T02 (vaccinated) groups showed less incidence of clinical signs. For the depression category, 100% of non-vaccinated pigs were affected and only 65% of vaccinated pigs. For general condition and respiratory distress, incidences of clinical signs were double (60%) in the T01 (non-vaccinated) group (60%) than T02 (vaccinated) group (30%). T02 (Vaccinated) pigs had significantly less virus in serum at all post-challenge sampling points (P< 0.05). Average daily gain during the 10 days post-challenge period was significantly higher in T02 (Vaccinated) than in T01 (Non-vaccinated) (P=0.0001)

Conclusion: This study demonstrates a protective vaccine effect in pigs vaccinated with Fostera® PRRS, showing a significant reduction of lung lesions, reduction in the clinical signs and significantly higher ADG in vaccinated pigs during the 10 days post-challenge phase.

Disclosure of Interest: None Declared

Keywords: PRRS MLV vaccine, PRRS RFLP 1-7-4

Vaccinology & Immunology

PO-PF3-084

Efficacy of Coglapix® in comparison to other EU licenced vaccines against Actinobacillus pleuropneumoniae infection

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Introduction: Porcine pleuropneumonia caused by *Actinobacillus pleuropneumoniae* (A.p.) is a highly contagious respiratory disease, characterized by rapid onset, short course, high morbidity and mortality. The disease occurs worldwide with varying incidence and severity. A.p. serotype 2 is still one of the most frequent serotypes in Europe. Controlling the disease is difficult, but vaccination can provide efficient protection by decreasing the mortality rate and also the prevalence and extension of pneumonia and pleuritis.
Coglapix® (Ceva) vaccine contains inactivated bacterium cells of serotype 1 and 2 A.p. strains and toxoids of Apx I, Apx II, and Apx III in order to provide protection against a broad range of A.p. serotypes. The aim of this study was to compare the efficiency of Coglapix® with four major A.p. vaccines licensed in the EU against (2 toxoid and 2 bacterin) in an experimental challenge model with A.p. 2.
Materials and Methods: Six weeks old pigs were vaccinated in a prime-boost regime three weeks apart either with Coglapix or its competitors' vaccines and were challenged with A.p. serotype 2 strain via aerosol with a dose of appr. 10^6 CFU/pig. After one week observation lung lesions and parietal pleura alterations were scored and the weighted scores (LSS) of the vaccinated groups were compared with ones obtained in un-vaccinated challenged group (positive control). Un-vaccinated non-challenged animals were used as negative controls.
Results: All vaccinated groups had significantly lower mean LLS values than the positive control group. The LLS figures did not differ statistically significantly among the vaccinated groups, however, the lowest value and corresponding highest calculated vaccine efficiency was recorded for the Coglapix® group, followed by 2 toxoid vaccines and then the 2 bacterin ones. Only Coglapix protected statistically significantly against mortality compared to the non-vaccinated control group. Mortality rates were 36% for the positive control, 0% for Coglapix®, 18% for each of bacterins, and 9% and 18% for the two toxoid vaccines, respectively.
Conclusion: The results confirmed the best efficacy of Coglapix®, a vaccine composed of both bacterin and Apx I-III toxoids, in comparison to four major EU-licensed A.p. vaccines against challenge with a virulent A.p. serotype 2.

Disclosure of Interest: None Declared

Keywords: Pleuropneumonia, vaccination

Poster Abstracts

Vaccinology & Immunology

PO-PF3-089

In vitro investigation on the compatibility of Coliprotec® F4, live oral vaccine against post-weaning diarrhoea (PWD) caused by F4-ETEC, and Aviblue®

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Introduction: Coliprotec® F4 is a live *E. coli* vaccine for immunisation of pigs against F4-ETEC, a prevalent cause of PWD. Coliprotec® F4 (Prevtec Microbia) can be administered via drenching or drinking water and should be consumed within 4 hours after reconstitution. In order to neutralize disinfectants in drinking water (i.e. chlorine), the addition of skimmed milk powder is recommended. Aviblue® (Elanco) is a stabilizer containing a blue colorant and is specifically formulated for drinking water in animals. It represents an alternative to skimmed milk powder to neutralize chlorine. This study investigates the stability of the Coliprotec® F4 vaccine when combined with Aviblue® at the recommended dose for drinking water administration using bowls or tanks and at high concentrations required for stock solutions used with dosing pumps.
Materials and Methods: Lyophilised Coliprotec® F4 vaccine was reconstituted in water and aliquots were mixed with chlorinated tap water containing Aviblue® at three final concentrations: Group 1X (two replicas, A and B), AviBlue® at 0.125 g/l (recommended minimum dosage in drinking water); Group 20X, AviBlue® at 2.5 g/l (stock solution concentration for dosing pumps at 5% injection rate); Group 50X, AviBlue® at 6.25 g/l (stock solution concentration for dosing pumps at 2% injection rate). The vaccine was added at a rate of 1 dose per 110-130 ml of final drinking water. Control groups using reverse osmosis (RO) water and tap water without Aviblue® were included. Viable cell counting was performed in all the groups at 0 (T0), 4 (T4) and 6 (T6) hours. Free chlorine in water was determined at the beginning of the experiment.
Results: No reduction in live bacterial counts was detected over the 6-hour tested period in the Aviblue® treated groups [Group 1X A: $T0=2.7x10^6$ CFU/ml; $T4=2.3x10^6$ CFU/ml; $T6=2.8x10^6$ CFU/ml. Group 1X B: $T0=2.1x10^6$ CFU/ml; $T4=1.9x10^6$ CFU/ml; $T6=2.0x10^6$ CFU/ml. Group 20X: $T0=5.7x10^7$ CFU/ml; $T4=5.9x10^7$ CFU/ml; $T6=5.4x10^7$ CFU/ml; Group 50X: $T0=1.3x10^8$ CFU/ml; $T4=1.8x10^8$ CFU/ml; $T6=1.3x10^8$ CFU/ml] though a significant reduction of vaccine viability was observed from T0 in the drinking water containing free-chlorine but no Aviblue®. The addition of Aviblue® did not affect the bacterial count compared to the RO water groups. Aviblue® protected Coliprotec® F4 strain from free-chlorine (0.5, 2.07, 0.4 and 0.72 ppm) present in tap water.
Conclusion: The results of this study indicate that Aviblue® at the recommended dosage in drinking water or high concentrations required for stock solutions for administration via dosing pumps does not affect the stability of Coliprotec® F4 and protects the vaccine against disinfectants, such as chlorine.

Disclosure of Interest: None Declared

Keywords: Post-weaning diarrhoea, vaccine, water stabilizer

Vaccinology & Immunology

PO-PF3-090

Induction of humoral immune response after perinatal or post-weaning immunization against PCV2

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Introduction: Vaccination against Porcine Cricovirus 2 (PCV2) is performed around weaning of piglets and provides robust protection against PCV2 disease. The current study was designed to test the hypothesis that the swine immune system was sufficiently mature shortly after birth and that vaccination would be equally efficacious when applied in the first week of life.
Materials and Methods: Fifty-seven colostrum-fed piglets of PCV2 vaccinated sows from a PCV2 positive barn were randomly placed into five treatment groups: early (6-7d: Early-VAC) and late PCV2 vaccinated (27-28d: Late-VAC), early (6-7d: Early KLH) and Late-KLH immunized (27-28d: Late-KLH), and control group (no treatment: Never-VAC). A single dose of Circumvent ® PCV Vaccine (Intervet Inc., Merck Animal Health) was used in this study. At 36-37 days of age (week 5), virus challenge (10E5 TCID50 i.n.) was administered to Early-VAC, Late-VAC and Never-VAC treatment groups. All pigs were serum PCV2 PCR negative prior to viral challenge. A KLH antibody ELISA was performed on the Early-KLH and Late-KLH serum samples. Saliva and individual serum samples were tested for the presence of PCV2 DNA via qPCR. Serum samples were assayed for neutralizing antibodies (NA) against PCV2. Wilcoxin and Kruskal-Wallis tests were applied to data sets with non-normal distributions and normal distributions were analysed using an ANOVA with post hoc Tukey-Kramer test (JMP®, version 10.0, SAS Institute).
Results: i. At 5 weeks after KLH immunization, both age groups had significant and similar KLH antibody titres ($P = 0.09$). ii. There was no difference in the serum PCV2-genome copy number in weeks 2-4 post-PCV2-challenge between Early-VAC, Late-VAC and Never-VAC. iii. Vaccination reduced salivary viral shedding relative to Never-VAC, although the Early-VAC group did have a phase of higher salivary viral shedding. iv. All treatment groups challenged with PCV2 showed a NA response across weeks 6 through 10. The Never-VAC treatment group was delayed by one week in mounting a NA response relative to both groups that were vaccinated.
Conclusion: This study provides evidence of humoral response induction to PCV2 vaccination in the first week of life, and confirms that the responses of early- and late-vaccinated piglets tend to converge over time. The current study adds evidence that exposure to a novel, non-pathogenic, antigen follows a similar time course. We suggest to vaccinate piglets early in life if the infectious challenge is likely to be experienced quite early in life.

Disclosure of Interest: None Declared

Keywords: Antibody interference, Neutralizing antibodies, PCV-2

Vaccinology & Immunology

PO-PF3-091

EFFICACY OF A NEW PCV2 AND M. HYOPNEUMONIAE COMBINATION VACCINE: COMPARATIVE FIELD STUDY VERSUS OTHER COMMONLY USED VACCINES

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Introduction: PCV2 and *M.hyopneumoniae* (Mhyo) are the most prevalent pathogens in finishing pigs, and are implicated in the Porcine Respiratory Disease Complex. Vaccination against PCV2 and Mhyo is standard practice in the pig industry, but a convenient ready-to-use one dose combination vaccine has not been available in Europe until now. **Here, field efficacy of such a new combination vaccine, Porcilis® PCV M Hyo, is described and compared against two commonly prescribed vaccines.**
Materials and Methods: **This study was performed according to a controlled, randomized and blinded design in a French swine farm**. Three week old piglets were randomly allocated, within litters, to one of three groups of ±166 piglets each. **Pigs in group A were vaccinated with Ingelvac M hyo and Ingelvac CircoFlex by two separate injections, while pigs in group B were vaccinated with Porcilis® PCV M Hyo by a single injection, and pigs in the control group were injected with saline.** The primary efficacy parameters were lung lesions at slaughter, PCV2 viremia and average daily weight gain (ADWG) during finishing. Secondary parameters were mortality, age to slaughter, average carcass weight and average turn-over per pig. The pigs were weighed at vaccination, at transfer to the finishing unit and before slaughter. Typical Mhyo lung lesions were scored at slaughter. Slaughter data were recorded for each included pig: date, age, carcass weight, and turn-over. PCV2 and Mhyo Elisa tests and quantitative PCV2 PCR were tested in serum samples from 45 pigs per group every 4 weeks. Differences were analyzed statistically.
Results: ADWG during finishing was A:868[a], B:881[b] and C:849[a] g/d. Frequency of pneumonia was A:85,5[a]; B:65,5[b] and C:82,6[a] % and average lesion scores were A:6,3[a]; B:3,1[b] and C:5,9[a]. Mortality was A:3,6[a]; B:6,0[a] and C:2,4[a] %.
Slaughter ages (A:185,6; B:184,5 and C:187,1 days), average carcass weights (A:91,0; B:91,6 and C:90,6 Kg) and average turn-over per pig (A:139,0 ; B:141,5 and C:139,6 €) were numerically better for group B.
Control pigs seroconverted for Mhyo and PCV2 at 14 weeks and were PCV2 viremic between 11 and 22 weeks. No PCV2 viremia was detected in groups A and B.
Conclusion: Control results indicate a clear PCV2 and Mhyo field challenge during this study. **Compared to the other vaccines, Porcilis® PCV M Hyo pigs had significantly better ADWG and Mhyo pneumonia lung lesions scores**. **Porcilis® PCV M Hyo had better numeric results for slaughter age, carcass weight, turn-over per pig.** The lack of significance may be the result of the relatively small number of study pigs. **In summary, Porcilis® PCV M Hyo is a safe, efficacious and convenient vaccine to protect against PCV2 and Mhyo infections.**

Disclosure of Interest: None Declared

Keywords: Mhyo, PCV2, Vaccine

Vaccinology & Immunology

PO-PF3-092

Suppression of host protein synthesis and regulation of interferon response by PRRS virus

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Introduction: Host innate immune system produces cytokines and chemokines in response to viral infection for protection. Type I interferons (IFN-α/β) are antiviral cytokines that play a major role during the early stage of infection, and have been shown to inhibit PRRSV replication. Thus, such cytokines are ideal targets for PRRSV to disarm and escape the host immune surveillance. Indeed, poor induction of proinflammatory cytokines and type I IFNs has been shown as the hallmark of PRRS virus in cells and pigs. Viral IFN antagonists been identified for PRRSV, and the nsp1 protein is a potent suppresser for IFN production. The objective of this study was to identify and characterize the molecular basis of the PRRS virus nsp1 protein-mediated innate immune modulation.
Materials and Methods: PRRSV nsp1 gene was subcloned to produce nsp1-alpha and nsp1-beta two subunits. Each subunit protein was expressed in cells and the modulation of IFN production was examined by reporter and bioassays. Host protein synthesis was determined by immunofluorescent microscopy and western blot, and host mRNA nuclear transport was determined by in situ hybridization using an oligo (dT) probe.
Results: The nsp1β protein was identified as the viral IFN antagonist that played the role for blocking host mRNA nuclear export. Blocking host mRNA nuclear export was determined to be common for different strains of PRRSV and was also identified for both North American genotype and European genotypes The inhibition of host mRNA nuclear export resulted in the suppression of host protein synthesis and was correlated to the suppression of IFN production and other antiviral proteins. This is a novel mechanism for host innate immune modulation and evasion by PRRSV.

Conclusion: PRRSV has the ability to escape the host innate immunity, and nsp1 is a potent viral antagonist suppressing the IFN production during infection. The nsp1α subunit of nsp1 is known to inhibit the formation of IFN enhanceosome and in the present study we show that the nsp1β subunit inhibits the host mRNA nuclear export and thus suppresses the production of IFNs and other antiviral proteins.

Disclosure of Interest: None Declared

Keywords: innate immunity, interferon, PRRS

Poster Abstracts

Vaccinology & Immunology

PO-PF3-100

Efficacy of Salmonella-based vaccine candidate expressing ApxIA, ApxIIA, ApxIIIA, ApfA and OmlA of Actinobacillus pleuropneumoniae in piglets

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Introduction: *Actinobacillus pleuropneumoniae* (APP) is an encapsulated respiratory pathogen of swine and the causative agent of porcine pleuropneumonia (PP). This disease results in large economic losses in the pig industry worldwide. Among several virulence exotoxins, Apx exotoxins are recognized as major virulence factors that play a predominant role in the pathogenesis of APP. In recent, it was reported that type IV fimbrial subunit protein ApfA was present in all these strains. Importantly, ApfA fimbriae are usually highly immunogenic. OmlA protein is a virulence factor of APP and has an important role in binding to transferrin to facilitate the acquisition of iron from the host. The objective of this study was to evaluate efficacy of the live attenuated *Salmonella* strains expressing recombinant ApxIA, ApxIIA, ApxIIIA, ApfA and OmlA antigens for prevention of PP in piglets.
Materials and Methods: Attenuated *Salmonella* strains expressing each ApxIA, ApxIIA, ApxIIA, ApfA and OmlA were conducted as vaccine strains. Cell pellets of vaccine candidate strains were resuspended to 2×10^9 colony-forming units (CFU)/mL in sterile PBS. Piglets were orally immunized with the live preparations on the same day. Twenty piglets were divided equally into two groups. All piglets were orally inoculated at 4 weeks of age [0 week post inoculation (WPI)]. Group A piglets were inoculated with 10 mL of sterile PBS. Group B piglets were immunized with approximately 2×10^{10} CFU in 10 mL of an equal-volume mixture of the five delivery strains. Blood samples were collected at 0, 2 and 4 WPI for the evaluation of immune response. All piglets were intranasally challenged at 4 WPPI with 2 ml of the mixture of the challenge strains. All challenged piglets were monitored daily for mortality and abnormal behavior until 7 days after the challenge.
Results: The expected sizes of ApfA and OmlA antigens were 15 and 38 kDa, respectively, and these bands were observed in the precipitated culture supernatants from the individual constructs. Serum IgG concentrations against the individual antigens in group B were significantly increased compared to those of group A from 2 WPI until the end of the study. Among 10 piglets of group A, 2 were dead within 7 days after challenge and the challenge strains were isolated from lung swab of 4 piglets with pneumonic lung in gross examination. Among 10 piglets of group B, however, the challenge strains were isolated from lung swab of only 2 piglets with pneumonic lung.
Conclusion: Overall, oral inoculation with our novel vaccine candidate can be considered an efficient protective immunization procedure against APP infection.

Disclosure of Interest: None Declared

Keywords: Actinobacillus pleuropneumoniae, Recombinant proteins; Immunization; Porcine pleuropneumonia; Vaccineation

Vaccinology & Immunology

PO-PF3-102

Vaccine efficacy of combined PCV2 and M. hyo vaccines against PCV2 challenge under laboratory conditions

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Introduction: Vaccination against PCV2 and Mhyo has become a standard measure in the swine industry worldwide. The objective of this study was to compare the efficacy of a freshly mixed PCV2 and M hyo combination and a pre-manufactured PCV2/M hyo vaccine combination against a PCV2b challenge.
Materials and Methods: The trial was conducted as a randomized, blinded vaccination-challenge efficacy study with 55 CDCD pigs. Animals were included at about 21 days of age vaccinated on D0, challenged with PCV2b on D14, and necropsied on D42.5 animals were kept as strict control group (non-vaccinated, non-challanged) 20 animals were not vaccinated and challenged on day 14 (challenge control group). 15 animals were vaccinated with FLEXcombo® (freshly mixed combination of Ingelvac CircoFLEX® and Ingelvac MycoFLEX® and challenged (Treatment group 1) and 15 animals were vaccinated with Foster PCV MH® and challenged on D14 (Treatment group2). Both products were used according to the manufacturer's instructions. After necropsy on day 42 animals were evaluated by three primary parameters: lymphoid depletion, inflammation of lymphoid tissues, and colonization of lymphoid tissues by PCV2. Individual weights were measured at animal inclusion, vaccination and challenge.
Results: The average lymph node score for lymphoid depletion, inflammation and immune histochemestry was significantly higher in the challenge control animals than in treatment group 1 and 2. Also significant differences ($p < 0.014$) were observed between treatment group 1 and 2 with lower scores in treatment group one for all 3 parameters. The average daily weight gain from challenge to necropsy was 432 g in the control group, 435 g in treatment group 2 and 499 g in treatment group 1.
Conclusion: The results of this study show that both vaccine combinations were efficacious in the control of PCV2 after challenge with a PCV2b strain 2 weeks after vaccination when comparing them with the challenge control group. However the results indicate that there are differences between the commercial PCV2/Mhyo vaccine combinations in terms of vaccine efficacy.

Disclosure of Interest: B. Grosse Liesner Conflict with: Boehringer Ingelheim, K. Kennedy Conflict with: Boehringer Ingelheim, M. Eichmeyer Conflict with: Boehringer Ingelheim, B. Fergen Conflict with: Boehringer Ingelheim

Keywords: PCV2, vaccine combination, vaccine efficacy

Vaccinology & Immunology

PO-PF3-103

Syringeability of Hyogen®, a vaccine against Mycoplasma hyopneumoniae

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Introduction: Pneumonia induced by M. hyopneumoniae is considered one of the most wide spread and most important chronic diseases in pigs. Over 70% of pig herds are vaccinated in countries with high developed swine industry. Mass vaccination is highly efficient in the protection against the development of bronchopneumonia but it is also demanding for the labor considering huge number animals to be injected. In this respect the easiness of the vaccine administration is important together of course with the safety and efficacy of the product. Hyogen® (Ceva) is an injectable inactivated M.hyo vaccine, proven efficient in preventing the lung lesions induced by M. hyo infection. The aim of the study was to assess the syringeability of Hyogen® in comparison with selected major competitor vaccines.
Materials and Methods: Hyogen® containing Imuvant™ as an adjuvant was compared with three commercial vaccines: Vaccine A containing Amphigen, vaccine B containing carbopol and vaccine C containing Impran as adjuvants. All products were measured at 5°C and ambient temperatures for syringeability in 21G, 19G and 18G needles. In all condition the glass syringe was filled in with the product and the time needed for injecting 10 ml volume under the constant pressure of 10N was measured in six replicates. The mean values were compared.
Results: At 5°C the Vaccine C was blocked in the needles (not injectable) irrespectively on the diameter. Hyogen ® and vaccine A had shorter cumulative mean time than Vaccine C ($p<0.05$). At ambient t° Hyogen® and Vaccine A had shorter cummulative mean time than Vaccine B or Vaccine C ($p<0.05$). Time required for Vaccine C exceeded 1min, 3min and 7min in 18G, 19G and 21G needles respectively.
Conclusion: Hyogen® together with Vaccine A proved to be best injectable M.hyo vaccine of four different commercial products tested. Vaccines B and C (with carbopol and Impran) required significantly longer time at both 5°C or ambient temperatures.

Disclosure of Interest: None Declared

Keywords: Mycoplasma hyopneumoniae, Syringeability, Vaccine

Vaccinology & Immunology

PO-PF3-123

Lactoferrin and its immunomodulatory properties

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Introduction: Lactoferrin (LF) is an iron-binding glycoprotein which belongs to a transferrin family. It is available as a commercial extract from bovine milk or colostrum and offers potential as a therapeutic intervention for modulating infections and intestinal pathologies. It was also used a feed supplement for piglest. To date, an ability of LF to bind LPS and then induce cells activation via TLR4 is known. LF itself, however, is belived to be able to activate cells via not yet well described pathway and can induce production of proinflammatory cytokines even without bound LPS. The aim of the study was to bring new information in this field.
Materials and Methods: In our experiment, porcine monocyte-derived macrophages (MDMF) or Human Embryonic Kidney cell line transfected with hTLR4A-MD2-CD14 (HEK4) were stimulated with purified LPS-free LF or with LF-LPS complex. After six hours, the cells were harvested and a cytokine transcript production was measured by qRT-PCR and the results were confirmed by Western blot analyses.
Results: In MDMF, transcription of proinflammatory cytokines and NFkappaBi molecule were induced even by LPS-free LF. Using HEK4 cell line, it was found that purified LPS-free LF did not induce any proinflammatory response. On the other hand, LF-LPS complex induced strong transcription of proinflammatory cytokines.
Conclusion: Our results suggest that LF is able not only to bind LPS, but LF itself may be a stimulant of proinflammatory response which is different than TLR4-mediated.
Supported by the Ministry of Agriculture of the Czech Republic (QJ1310258) and MEYS (project LO1218 under NPU-I program).

Disclosure of Interest: None Declared

Keywords: lactoferrin, lipopolysaccharide, Macrophage

Poster Abstracts

Vaccinology & Immunology

PO-PF3-126

Intranasal vaccination: a comparison of a new nozzle versus administration via syringe

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Introduction: The intranasal administration is a routine way for administering vaccines to young pigs. There is a need for better administration devices that enable a consistent dispersion of the products in the upper respiratory tract.
Materials and Methods: Ten 3-5 healthy pigs were used to investigate the distribution of a blue dye (McCormick® Bright Blue Food Color) given intranasally either by normal syringe or a nozzle that has been tailor made to fit on a vaccine "gun" (Primatech, Neogen Corporation). One ml per nostril was given to 8 pigs via the nozzle and two animals received 1ml per nostril per 2ml plastic syringe.
Results: Administering 1 ml per nostril with the nozzle attached to the vaccine "gun" lead to a consistent distribution of the blue dye in pigs and colored the upper respiratory tract up to the primary bronchi including the tonsils, whereas administrating the dye via a syringe resulted in distribution up to the bronchioli and into the lung tissue.
Conclusion: Administration of fluids via the new nozzle resulted in a more consistent distribution compared to administering the fluid via a syringe. Using the nozzle may result in less aspiration pneumonia as it can be anticipated with using a vaccine. Using the nozzle with commercial vaccines licensed for intranasal administration need to be conducted to confirm this observation.

Disclosure of Interest: None Declared

Keywords: dispersion, intranasal, nozzle

Vaccinology & Immunology

PO-PF3-135

Comparative study of the humoral immune responses developed by 5 commercial monovalent Erysipelothrix rhusiopathiae vaccines.

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Introduction: The aim of this study was to compare the humoral immune responses elicited in naïve pigs by five different inactivated monovalent *Erysipelothrix rhusiopathiae* vaccines.
Materials and Methods: A controlled, blinded field study was performed on a 360-sow farm located in Catalonia (Spain). A total of one hundred and eighty 13-week-old pigs, clinically healthy and free from antibodies against *E. rhusiopathiae*, were randomly assigned to six different groups. Animals from groups 1 to 5 were immunised twice i.m. (2 ml/dose, at 13 and 16 weeks of age approx.) with five different commercially available vaccines in Europe. Group 1 was immunised with ERYSENG®, a vaccine adjuvanted with Hipramune® G^d, and groups 2-4 were immunised with three different swine erysipelas monovalent vaccines. Animals from the control group (Group 6) received a phosphate buffered saline (PBS) following the same schedule. 15 serum samples/group were obtained on days 0, 19, 47 and 78 and antibodies to *E. rhusiopathiae* (IgG) were titrated using a commercially available ELISA (CIVTEST® SUIS SE/MR). Samples were considered positive when IRPC ≥ 40. Antibody titres were compared between groups using the Kruskall-Wallis test (p<0.05).
Results: *E. rhusiopathiae* antibody titres from group 1 (ERYSENG®) were the highest throughout the fattening period and showed statistically significant differences to groups 2, 4 and 5 on day 19 and groups 2 and 5 on day 47 (p<0.05). A decrease in the mean *E. rhusiopathiae* titres for all vaccinated groups was observed from day 47 onwards. At the end of the study (day 78) the group vaccinated with ERYSENG® was the only one which showed statistically significant differences to the control group. The control group remained negative and stable during the entire fattening period.
The percentage of seropositive pigs in the ERYSENG® group was the highest of all the different commercial vaccines during the entire study. At the end of the fattening period, around 80% of the animals vaccinated with ERYSENG® were seropositive, with titres above the cut-off (IRPC ≥ 40). While the percentage of seropositive samples from the other groups was: 40% (group 2), 46% (group 3), 22% (group 4) and 60% (group 5).
Conclusion: The humoral immunity elicited by ERYSENG® was faster, higher and lasted longer than the humoral immune response developed by the other commercial vaccines. ERYSENG® provided the best humoral protection during the entire fattening period.

Disclosure of Interest: None Declared

Keywords: Eryseng, Erysipelothrix rhusiopathiae, Swine erysipelas

Vaccinology & Immunology

PO-PF3-136

Effect of azaperone administration after farrowing on maternally derived immunoglobulin level in suckling piglets using the Immunocrit® method

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Introduction: The dramatic change in sow prolificacy observed in the modern swine industry over the last decade was also accompanied by a significant increase in pre-weaning piglet mortality. This emphasizes the importance to guarantee maximum colostrum intake within the first hours after birth as a readily available energy source and for passive immunity, increasing the piglets chances for survival and optimal performance. Recent studies show that the use of azaperone in sows at the end of parturition may improve piglet performances. Recently, a new assessment technique for the piglet's maternally derived immunoglobulin level has been developed and evaluated. The objective of the trial was to investigate the effect of sow azaperone treatment at parturition on the maternally derived IgG level using the Immunocrit® method.
Materials and Methods: The trial was performed in a 400 sow farrow-to-finish herd based in The Netherlands. Upon parturition, sows (n=80) were randomly assigned to one of both trial groups, namely control group (C-group) or azaperone group (A-group). The sows in the A-group were injected with 8 ml of azaperone (Stresnil, Elanco Animal Health) upon the end of parturition. For analytical purposes, 6 piglets in each litter were selected, based on their life weight on day 2, and subsequently bled. Blood was further processed as described previously with the increase of centrifugation to 10 minutes instead of 5 and subsequent immunocrit analysis was performed. Results were statistically analysed using SAS for significant effects between treatment groups.
Results: Results of the immunocrit immunoglobulin analysis showed a significant increase in IgG levels (35.02 mg/ml in C-group vs. 36.14 mg/ml in A-group) in piglets born from sows injected with azaperone following the end of parturition. A significant decrease in mortality could be observed in the litter with azaperone treatment at the end of parturition (13.75% in A-group vs. 17.41% in C-group). Piglets which finally died has a much lower (- 8 to 11 mg/ml) IgG level in their blood at first sampling as compared to surviving piglets at weaning.
Conclusion: Using the new immunoglobulin analysis technique, Immunocrit®, a clear idea could be obtained of the colostrums distribution in treated and untreated litters. The results are in accordance with other studies showing an improved colostrum IgG distribution in azaperone treated litters. Based on earlier research, the decision could be taken to treat sows at risk with azaperone, in order to obtain a more evenly distributed colostrum IgG level among the piglets in the litter.

Disclosure of Interest: None Declared

Keywords: azaperone treatment, blood IgG level, piglet mortality

Vaccinology & Immunology

PO-PF3-137

Histopathologic evaluation of abnormal meat induced by foot-and-mouth disease vaccination

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Introduction: In 2010, three outbreaks of foot-and-mouth disease (FMD) occurred in South Korea. Among these outbreaks, the largest occurred from November 2010 to April 2011 and induced 153 total cases occurring in both cattle and pigs. During this outbreak, a nationwide vaccination policy was enforced and carried out in susceptible animals, including pigs. However, local reactions at the injection site were present after vaccination. Even now, these local reactions are an important cause of economic loss for the Korean pork industry. The aim of this study was to evaluate the histopathologic features of injection sites after FMD vaccination at multiple time periods.
Materials and Methods: 60-day-old 156 domestic pigs were injected intramuscularly with 2mL of an oil-adjuvanted FMD vaccine. The animals were processed at 7, 30, 60, 90, or 135 days in a slaughterhouse. After gross examination, tissues of the injection site were fixed in 10% buffered formalin. The fixed samples were embedded in paraffin and stained with hematoxylin and eosin (H&E) stain for histopathologic analysis.
Results: Histopathologically, the injection sites showed varying degrees of an inflammatory myopathy with intralesional, clear, round vacuoles. At 7 days after vaccination, all animals showed severe local inflammatory reactions with a mixed population of inflammatory cells containing polymorphonuclear and mononuclear cells. Muscle fibers in the region of the injection were atrophied or degenerated. At 30 to 60 days after vaccination, typical granulomatous and pyogranulomatous inflammatory lesions were observed in most of the samples. Many clear vacuoles were phagocytosed by multinucleated giant cells. Focal intralesional mineralization and abscessation were also found. At 90 days after vaccination, granulomatous lesions decreased slightly. At 135 days after vaccination, partial replacement of adipose tissue was observed within the muscle of the injection site.
Conclusion: Formation of abnormal meat at the injection site was the result of various inflammatory reactions that also lead to muscular degeneration. Clear vacuoles within the lesion probably represented the oil adjuvant used in the FMD vaccine. Most granulomatous inflammatory reactions were observed within the periphery of vacuoles. Although the degree of typical granulomatous lesions decreased at 90 days after vaccination, focal microscopic lesions persisted at the injection site until 135 days post-injection. Based on this analysis, we confirmed that the increased occurrence of abnormal meats was associated with FMD vaccination in pigs.

Disclosure of Interest: None Declared

Keywords: abnormal meat, FMD vaccine, histopathology

Poster Abstracts

Vaccinology & Immunology

PO-PF3-144

Efficacy evaluation of CSFV E2-PCV2 ORF2 bivalent subunit vaccine in pigs

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Introduction: Live attenuated LPC vaccine is routinely utilized in pig farms for control of classical swine fever (CSF) outbreak in Taiwan. However, the efficacy of LPC vaccine might be impaired by interference of maternal antibody derived from sow and concurrent porcine circovirus disease (PCVD) infection in piglets. Recently, it illustrated that the provoked CSFV-specific antibody after LPC immunization showed significant retardation and prompt declining in PCVD infected pigs. To prevent the impact of both diseases in post-weaning stage, a bivalent vaccine combined with recombinant subunit PCV2 ORF2 and CSFV E2 antigens may provide a good strategy to elicit good protective immune responses against both diseases due to sharing of similar immunization schedule and reducing stress from repeat vaccination in piglets.
Materials and Methods: A prototype of bivalent CSFV E2/PCV2 ORF2 subunit vaccine was applied for twice immunization in piglets at 4 and 6 weeks old in a conventional pig farm. The both provoked specific immune responses were evaluated by PCV2 IFA assay and neutralizing antibody/ELISA (IDEXX) titer against CSFV. In addition, the protective efficacy was determined by artificially challenged with 2×10^5 $TCID_{50}$ of PCV2 at 8 weeks old for monitoring average daily weight gain (ADWG) and PCV2 viral load in serum. Four weeks after inoculation, all challenged pigs were performed necropsy for pathological examination and further evaluated by immunohistochemical (IHC) assay and viral load detection in lymph nodes.
Results: In determination of vaccine efficacy and duration of immune responses, all vaccinated piglets showed increasing of neutralizing antibody titers against CSFV to 1:256 and exhibited significantly higher blocking percentages by the blocking ELISA (IDEXX) with average of 76±0.8% than the placebo group of 15±2.6% ($p<0.05$). In addition, all immunized pigs seroconverted to PCV2-specific antibody with average IFA titer of Log_2 13.3±0.63 in contrast to 10.9±0.74 of the control group ($p<0.05$).
In PCV2 challenge trial, the results displayed significant difference on ADWG between vaccinated and control groups (795±29g vs. 385±131g; $p<0.05$). Furthermore, both quantitative PCR and IHC detection also revealed the bivalent vaccine could reduce PCV2 viral load in serum and viral distribution in peripheral lymph nodes after challenge.
Conclusion: The results indicated that the bivalent CSFV E2/PCV2 ORF2 vaccine showed good safety and could elicit good protective immunity against both diseases in conventional pigs. Moreover, all vaccinated pigs could significantly improve the ADWG and reduce viral load both in serum and lymph nodes after PCV2 challenge.

Disclosure of Interest: None Declared

Keywords: Bivalent vaccine, CSFV, PCV2

Vaccinology & Immunology

PO-PF3-152

Vaccination with an innovative pressure-adjustable needle-free injection device

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Introduction: The Valery® (Giordano Poultry Plast, Italy) is an innovative pressure-adjustable needle-free injection device (NFID). The objective of this study was to determine appropriate operation of this NFID for vaccination by evaluating the location of a vaccine in pigs after application.
Materials and Methods: In a first trial, 4 groups of 12 pigs weighing respectively 4-5 kg, 6-7 kg, 8-10 kg and 15-20 kg were injected with a dye-labeled vaccine (Circovac, Merial), 0.5 mL in the neck using the NFID. Per group 3 pressure settings of the device were tested (A:low, B:medium or C:high), 4 pigs each. The pigs were immediately euthanized and frozen in vertical position (48 h; -20°C). Cross-sectional slices (3 per pig, 1 cm thick) at the injection sites were collected and digitalized by photography. The slices were checked on the penetration and dispersion of the vaccine by image analysis.
In a second trial, 2 groups of 4 pigs weighing respectively 6-7 kg or 8-10 kg were used. Following cleaning and drying of the skin surface, the vaccine was injected using the NFID, in the neck. Before injection a piece of blotting paper in an empty screw cap tube had been weighed. Just after injection, the piece of paper was applied on the skin surface at the injection site for 2 secs , then stored in the screw cap container. The tube was weighed. The percentage of vaccine dose on the skin surface (SkQ) was calculated by difference
Results: The depth of penetration of the vaccine whatever the weight group or pressure settings was 2.33±0.76 cm (n=48) with no difference observed between the pressure settings (A, n. 16, 2.14±0.77; B n. 16, 2.61±0.72; C, n. 16, 2.23±0.75 cm) or the weight group (4-5Kg, n.12, 2.18±0.4; 6-7Kg, n. 12, 2.40±0.24; 8-10Kg, n. 12, 1.89±0.56; 15-20Kg, n. 12, 2.83±1.18 cm) . The percentage of the vaccine present at the muscular level was varying between 70% (pressure A, 4-5kg) to 100% (pressure C, 6-7 Kg). In the different weight groups on average 87.0%. Setting B and C had the highest amount IM, resp. 92.5 and 89.2%. The area of muscular distribution is the highest with pressure C compared to A and B (p=0.04).
In trial 2,the SkQ was low whatever the operating pressure (A: 3.5±2.2%; B: 2.9±1.9%; C:1.5±0.4%) and the weight groups with a significant inverse relation between operating pressure and SkQ as well as a remarkable uniformity at the highest pressure setting.
Conclusion: Under the conditions of the study, the Valery NFID was shown to deliver a 0.5 mL vaccine recommended for IM vaccination satisfactorily. It is advised to use pressure settings medium to high. The volume of vaccine spread on the skin was considered as acceptable. Vaccination compliance was thought not to be impacted by the NFID.

Disclosure of Interest: G. Sarli: None Declared, C. Bianco: None Declared, S. Paranese: None Declared, F. Ostanello: None Declared, D. Lorini Conflict with: Giordano Poultry Plast S.p.A., O. Merdy Conflict with: Merial S.A.S., F. Joisel Conflict with: Merial S.A.S., H. Smits Conflict with: Merial B.V.

Keywords: Compliance, needle free

Vaccinology & Immunology

PO-PF3-169

High levels of Maternal Derived Antibodies (MDA) against PCV2 influences seroconversion due to vaccination, a case report.

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Introduction: PCV2 is a widespread and important disease in pigs. Commercially available vaccines are successful in reducing clinical damage caused by PCV2.
In this case report, PDNS and high mortality were reported in finishers that were vaccinated against PCV2.
This case is situated in a closed herd in the Netherlands with 950 sows, 300 replacement gilts and 650 finishers. The farm is well managed and has high hygienic standards. At the end of august 2014, despite vaccination against PCV2, PDNS cases and high mortality (5-10%) were reported in the finishers. Symptoms started at 14-16 weeks of age. Diagnosis of PCV2 disease was made by autopsy and positive IHC (immunohistochemistry) of the inguinal lymphnodes.
Previous investigations in the farm revealed that offspring of gilts had higher levels of Maternally Derived Antibodies (MDA) then offspring of sows. As literature describes that high levels of MDA can interfere with serological response to vaccination, the PCV2 vaccination was postponed to 6 weeks of age.
Materials and Methods: Vaccination technique and vaccine storage were evaluated.
To evaluate vaccination response, in total 20 pigs, 10 pigs born from gilts and 10 pigs born from sows, were individually followed in time. The pigs were bled 3 times: at approximately 21 days of age, before vaccination at the age of 6 weeks and 3 weeks after vaccination.
The serum samples were tested with a Immunoperoxidase Monolayer Assay (IPMA) test at the lab of Ghent University.
Pigs at risk of having a high level of MDA were given a second vaccination at the age of 10 weeks.
Results: No abnormalities were found in vaccination technique or vaccine storage. Offspring of gilts had an average IPMA log2 titer of 17.32 at the time of vaccination, and 3 out of 10 showed seroconversion after vaccination. Offspring of sows had an average IPMA log2 titer of 13.92 at the time of vaccination and 8 out of 10 showed seroconversion after vaccination.
After introducing a second vaccination for offspring of gilts, PCV2 symptoms rapidly reduced.
Conclusion: When PCV2 associated symptoms appear despite a proper vaccination against PCV2, one should be aware of the possibility that MDA can interfere with the effect of vaccination. A high percentage of the affected finishers was offspring of gilts, and had high levels of MDA. Postponing the vaccination and searching for the source of high MDA (circulating virus or PCV 2 vaccination of sows) can solve the problem.

Disclosure of Interest: None Declared

Keywords: PCV2, MDA, vaccination

Vaccinology & Immunology

PO-PF3-170

Intradermal vaccination (Hipradermic®) with UNISTRAIN® PRRS in a mass vaccination in sows

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Introduction: The intradermal (ID) route for mass vaccination has become established as an alternative to the intramuscular (IM) route because of the improvement in animal welfare, an effective immune response and ease of administration, amongst other reasons. The MLV UNISTRAIN® PRRS can be applied both by IM injection and by ID injection with Hipradermic® (a needle-free injection device). The aim of this study was to demonstrate that UNISTRAIN® PRRS applied via the ID route (Hipradermic®) was as safe and efficacious as when applied via the IM route in a mass vaccination in sows.
Materials and Methods: A PRRS-positive farm with 1,400 sows, following a PRRS vaccination protocol of 4 mass IM vaccinations per year, was mass vaccinated ID with UNISTRAIN® PRRS using Hipradermic® (0.2 ml/dose). Thirty-four sows from the same farm were vaccinated IM, also with UNISTRAIN® PRRS (2 ml/dose). The 34 animals vaccinated IM together with 34 animals vaccinated ID were individually identified for evaluation of the safety and efficacy of the vaccine. The efficacy of the vaccine was assessed by the PRRSV antibody response by ELISA (IDEXX PRRS x3 and Civtest Suis PRRS E/S) and viraemia by SYBR Green RT-PCR in serum samples prior to vaccination and at 28 days post-vaccination (dpv). In addition, the local reaction, body temperature and reproductive parameters (born alive and stillborn piglets) were evaluated at individual level. Finally, the reproductive parameters at the first farrowing post-vaccination were compared with the previous data in all sows. Different statistical tests were performed according to the recorded data.
Results: No presence of RNA of PRRSV was detected at 28 dpv in any of the groups (IM and ID), although there could have been some undetected replication at very low levels. Independently of the ELISA test, the PRRS antibody values at 28 dpv were higher than before vaccination (t-test, $p<0.05$) by the different routes, however no significant differences were detected. After ID administration, local reactions (inflammation and/or redness) were observed, which were mild and transient, resolving within 2 days. Similar reproductive values were obtained pre- and post-vaccination at individual and farm level, as well as in the comparison between the IM and ID vaccinated animals (Mann Whitney and Friedman Test, $p>0.05$).
Conclusion: The results obtained showed that UNISTRAIN® PRRS injected via the ID route with Hipradermic® in sows induced similar antibody levels at 28 dpv, whilst safety and reproductive parameters were comparable to the traditional IM route, which suggests similar efficacy against a PRRS outbreak.

Disclosure of Interest: None Declared

Keywords: intradermal vaccination, mass vaccination, PRRS MLV vaccine

Poster Abstracts

Vaccinology & Immunology

PO-PF3-171

Feeding a Lactobacillus acidophilus fermentation product attenuates the acute phase response following a proinflammatory challenge in young pigs

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Introduction: In the pig, the acute phase immune response can be stimulated with lipopolysaccharide (LPS), a molecule located on the outer membrane of gram-negative bacteria. Stimulation with LPS induces the expression of proinflammatory cytokines. Various species of *Lactobacillus* may reduce the proinflammatory response to pathogenic bacteria and enhance innate immune function. This study was designed to determine if feeding a *Lactobacillus acidophilus* fermentation product to weaned pigs would reduce stress and acute phase responses (APR) following a proinflammatory challenge with LPS.
Materials and Methods: Pigs (n = 30; 6.4 ± 0.1 kg BW) were housed individually in pens with *ad libitum* access to feed and water. Pigs were weighed upon arrival, assigned to 1 of 3 groups (n=10/treatment), and fed for 18 d: 1) Control, fed a non-medicated starter diet; 2) Control + *Lactobacillus acidophilus* fermentation product at 1 kg/MT (SGX1; Diamond V SynGenX™, Cedar Rapids, IA), and 3) Control + *Lactobacillus acidophilus* fermentation product at 2 kg/MT (SGX2). Pigs were anesthetized on d 7 and 14 for insertion of an i.p. temperature device and jugular catheter, respectively. On d 15, pigs were challenged i.v. with LPS (25 µg/kg BW). Blood samples were collected at 0.5 h (serum) and 1 h (complete blood cell counts) intervals from -2 to 8 h and at 24 h relative to LPS administration at 0 h. Pigs were weighed on d 7, 14, and 18, while feeders were weighed on d 7, 11, 14, 17, and 18.
Results: There was a treatment × time interaction ($P < 0.01$) for pig BW and ADG. The SGX1 pigs had the greatest body weight at 7, 14, and 18 d. Pig ADG was greater in SGX1 and SGX2 on d14, yet was less on d 18 compared to Control. In response to LPS, there was a greater change in IP temperature in Control pigs compared to SGX1 and SGX2 pigs ($P < 0.01$). There was a treatment × time interaction ($P=0.006$) for cortisol; SGX2 pigs had decreased cortisol from 2.5 to 4.5 h and at 5.5 and 6.5 h compared to SGX1 and/or Control pigs. White blood cells, neutrophils and lymphocytes were decreased in SGX1 and SGX2 compared to Control pigs ($P < 0.001$). There were treatment × time interactions for TNF-α, IFN-γ and IL-6 ($P \leq 0.04$). Specifically, SGX1 pigs had a decreased ($P \leq 0.04$) TNF-α response while SGX2 pigs had a greater ($P \leq 0.01$) response. The IFN-γ response was delayed and decreased in SGX2 pigs compared to Control and SGX1 pigs ($P \leq 0.02$). The IL-6 response was decreased in both SGX1 and SGX2 compared to Control pigs ($P \leq 0.01$).
Conclusion: These data demonstrate that feeding a *Lactobacillus acidophilus* fermentation product to weaned pigs can attenuate the APR to an LPS challenge.

Disclosure of Interest: None Declared

Keywords: acute phase response, Lactobacillus acidophilus fermentation product, lipopolysaccharide

Vaccinology & Immunology

PO-PF3-173

Current vaccines provide insufficient protection against NADC-30 like strain of PRRSV novel emerged in China

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Introduction: Porcine reproductive and respiratory syndrome reproductive virus infection causes reproductive failure in sows and respiratory disorder in all age of pigs. This pathogen is characterized as genetic, antigenic and biological variation, which leads extreme diversity in the clinical phenotypes and severities induced by the PRRSV infection. The variation and limitation of modified live vaccine on cross-protection against heterologous variants seriously handle the effectivity of disease prevention. Recently an emergence of a novel PRRSV (NADC30-like) in China that is genetically similar to the NADC30 strain isolated in the United States in 2008 was reported. However, the pathogenicity of this novel NADC30-like virus and weather the current commercial vaccine could provide sufficient protection against this kind of virus are still unknown.
Materials and Methods: 6 landrace SPF piglets at 42-day-old were intranasally inoculated with 2 ml CHsx1401 virus ($2x10^5$ TCID). And the pathogenicity of this virus was investigated through febrile response, clinical scores, average daily weight gain, viremia, PRRSV specific antibody level, gross and microscopic lung lesions and immunohistochemistry (IHC) examination.
As well, 30 SPF piglets at 21 days of age were randomly divide into 5 groups and piglets in 3 groups were vaccinated with commercial vaccine and attenuated low pathogenic strains respectively, and then the vaccinated and mock piglets were inoculated with 2 ml CHsx1401 virus ($2x10^5$ TCID) at 28 day post vaccination. Then the cross-protection efficacy was evaluated according the same items above.
Results: Febrile responds and obvious clinical signs were observed inCHsx1401 group. And the ADWG of CHsx1401 group was 0.12 kg less than that of control both on 14 DPI and 21 DPI. And the gross and microscopic lesions scores and IHC scores of CHsx1401 groups were significant higher than that of control as well.
Post CHsx1401 challenge, the febrile responds and clinical signs were still observed in all vaccinated groups. And all vaccinated groups had significantly lower viral titer than that in mock group at last two weeks indicated the vaccination partially provide benefit for viremia clearance in sera. Finally the immunization with current commercial vaccines provided little effector on reducing the gross and microscopic lung lesion in piglets challenged with NADC30-like virus.
Conclusion: NADC30-like virus CHsx1401 is a moderate virulent strain and current commercial vaccine provides insufficient protection against this virus, which play limited roles on either alleviating the clinical symptom nor reducing the gross and microscopic lung lesions.

Disclosure of Interest: None Declared

Keywords: pathogenicity, Porcine reproductive and respiratory syndrome virus (PRRSV), vaccine protection

Vaccinology & Immunology

PO-PF3-181

S. Typhimurium and S. Typhimurium Monophasic variant attenuated vaccines. A comparison of efficacy in homologous and heterologous infection in piglets

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Introduction: *Salmonella* Typhimurium and its monophasic variant (*S.* Typhimurium 1, 4, [5], 12:i-) are increasingly responsible of food borne infections in humans and pork represents the principal source of infection. Infection is generally sub-clinical in pigs and carrier pigs could introduce bacteria in the slaughterhouse. The aim of the study was to test the efficacy and safety of an attenuated vaccine of S. Typhimurium 1, 4, [5], 12:i- (*S.* Typhimurium Monophasic variant ΔznuABC) during an homologous and heterologous infection, with a field isolated strain of *S.* Typhimurium. The efficacy and safety of *S.* Typhimurium Monophasic variant ΔznuABC was compared to an attenuated strain of *S.* Typhimurium (*S.* Typhimurium ΔznuABC).
Materials and Methods: Twenty eight weaned piglets were divided in 3 groups and acclimatized for a week. Group T was composed of 8 piglets vaccinated with an oral administration of *S.* Typhimurium ΔznuABC at the final dose of 5 x 10^7 CFU. Group M was composed of 10 piglets vaccinated with an oral administration of *S.* Typhimurium Monophasic variant ΔznuABC at the final dose of 5 x 10^7 CFU. Group C was composed of 10 unvaccinated piglets. At day 35 after vaccination, all piglets were challenged by an oral gavage with 5 x 10^8 CFU of *S.* Typhimurium Monophasic variant or *S.* Typhimurium. Particularly, piglets from group T were divided in 2 groups: 3 piglets were infected with *S.* Typhimurium, the other 5 piglets were infected with *S.* Typhimurium 1, 4, [5], 12:i-. Piglets from group M were divided in 2 groups: 5 piglets were infected with *S.* Typhimurium. The other 5 piglets were infected with *S.* Typhimurium 1, 4, [5], 12:i-. Unvaccinated piglets were divided in 2 groups: 5 piglets were infected with *S.* Typhimurium, the other 5 piglets were infected with *S.* Typhimurium 1, 4, [5], 12:i-. Analyzed parameters were weight, temperature, fecal shedding and organ colonization.
Results: In control groups, the amount of *S.* Typhimurium in feces tends to be higher than *S.* Typhimurium 1, 4, [5], 12:i- from challenge to the end of the trial and temperature was significantly different at day 1 after infection indicating that *S.* Typhimurium was more virulent than *S.* Typhimurium 1, 4, [5], 12:i-. The safety of vaccine strains was monitored analyzing fecal shedding and growth of animals.
Conclusion: Attenuated vaccines were safe, in fact they were not isolated in feces after three weeks from vaccination and did not affected growth of animals. Furthermore, both attenuated vaccines reduced the shedding of virulent strains in comparison to unvaccinated groups and *S.* Typhimurium ΔznuABC appeared more effective in homologous and heterologous challenge infections.

Disclosure of Interest: None Declared

Keywords: attenuated vaccines , S. Typhimurium , S. Typhimurium 1, 4, [5], 12:i-

Vaccinology & Immunology

PO-PF3-183

Efficacy of vaccination with a mixed injection of RHINISENG® and SUISENG® in sows

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Introduction: The objective of this study was to evaluate the efficacy of combined vaccination with RHINISENG® and SUISENG® in sows by evaluating the immunity transmitted via colostrum to the piglets.
Materials and Methods: It was performed on a 1,000 sow farm in France. 3 batches were included: B1 and B2 were sows and B3 were gilts. RHINISENG® + SUISENG® were administered i.m. with a 4 ml/dose and the vaccination protocol was: sows from B1 and B2 received a primary vaccination 6 and 3 weeks before farrowing, and gilts from B3 received a primary vaccination in quarantine, with two doses 3 weeks apart, and a booster dose 3 weeks before farrowing.
To monitor the efficacy of this vaccination, serological samples from 25 sows were taken 12 days after farrowing: 10 animals in B1 and B3, and 5 animals in B2. Serum samples were taken from 3 piglets randomly selected from each sow at 12 days of age from all batches, and the same animals from groups B1 and B3 were monitored and sampled at 8, 13 and 17 weeks of age. The following serological analyses were conducted: firstly, detection of antibodies against the PM toxin using a commercial ELISA (IDEIA™ pasteurella multocida toxin, OXOID**).** Secondly, 4 in-house ELISAs (HIPRA) were used to detect the antibodies against different attachment factors of *E.coli*: F4ab, F4ac, F5 and F6. Moreover, lung and nasal turbinate lesions in the slaughterhouse after vaccination were compared with historical data of the farm, according to the IFIP method.
Results: All the sows were positive for PMT and *E. coli* attachment factor antibodies 12 days after farrowing. For piglets, at 12 days of age, 100% were positive for antibodies against PMT and 98.5% of them were also positive against the attachment factors of *E. coli.*
Although the percentage of positive piglets against PMT declined with age, protection lasted for more than 8 weeks. There was a greater difference between piglets in B1 and B3: at 13 weeks of age, 21% of the pigs in B1 were protected, whilst 48% of the pigs in B3 were positive. This difference may be related to the fact that sows in B1 received two doses, whereas gilts in B3 received a three doses.
Finally, comparing before and after vaccination, it was observed a reduction of lungs and nasal turbinate lesions in the slaughterhouse from 3.80 to 1.07, and from 4 to 1.68, respectively, according to the IFIP method.
Conclusion: This study shows that immunization of sows with the combination of RHINISENG® and SUISENG® provides an effective humoral passive immunity to their piglets (at 12 days of age 100% of animals were positive against PMT and 98.5% against the attachement factors of *E. coli*) and, consequently, reduces the lung and nasal turbinate lesions at the slaughterhouse.

Disclosure of Interest: None Declared

Keywords: atrophic rhinitis, Neonatal diarrhoea, vaccine combination

Poster Abstracts

Vaccinology & Immunology

PO-PF3-186

Local reactions after vaccination detected via magnetic resonance imaging and compared with pathomorphological examination

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Introduction: Local reactions are possible side effects after vaccination in animals. In the case of live viral vaccines, local reactions are mostly small and transient, whereas in the case of inactivated vaccines local reactions are often more pronounced. These effects are due to the use of adjuvants in inactivated products. Depending on the adjuvant type, local inflammatory reactions at the injection site are common but vary in extent. In Europe the safety of veterinary vaccines is assessed in clinical trials, which use pathologic examinations to describe the extent of the local reaction.
Materials and Methods: In order to reduce the number of animals in clinical trials, this study aimed to assess the local tissue reaction after vaccination by repetitive magnetic resonance imaging (MRI) at the live animal, which offers the possibility of repeated examinations in the same animal over time. The present study evaluated the extent of local tissue reaction at day 1, 3, 8, 15, 22 & 29 after vaccination via MRI and compared these findings with histopathology of the injection site at day 29 after vaccination. A total of 32 piglets, divided into two examination groups (16 piglets each), were injected into marked injection sites with one of two inactivated commercial vaccines (with *Mycoplasma hyopneumoniae* antigen). All animals were sedated and scanned repeatedly by MRI up to day 29 after vaccination. 50% of the animals of each examination group (8 piglets each) received a contrast agent during all MRI scans. These eight animals per group were euthanized at day 29 after vaccination and underwent a pathomorphological examination.
Results: The repeated MRI examinations showed different extents (e.g. from 0.09 ± 0.04 cm^3 for group I to 0.37 ± 0.14 cm^3 for group II, for the contrast agent MRI sequence at day 29) of local reaction between both groups. The comparison of the MRI results with the pathomorphological findings at the injection site yielded matching results concerning the sizes of the affected tissue volumes or areas (e.g. R^2 = 0.56 between MRI extent and maximum distribution of local reaction using histopathology at day 29).
Conclusion: This study shows that (1) MRI can describe the local reaction after vaccination in the live animal repetitively, (2) different vaccines show different extents of local reactions and (3) it seems that for regulatory safety testing the number of animals can be reduced to 8 animals per examination group. Complementary a final pathomorphological examination allows the identification of the kind and local distribution of the reaction.

Disclosure of Interest: None Declared

Keywords: local reaction, magnetic resonance imaging, vaccine safety

Vaccinology & Immunology

PO-PF3-191

Effects of two different Circovirus type 2 and Mycoplasma Hyopneumoniae vaccines mixture on acute phase proteins in Iberian piglets

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Introduction: Porcine circovirus type 2 (PCV2) and co-infection with *Mycoplasma hyopneumoniae* (M.hyo) plays a primary role in the porcine respiratory disease complex. Acute phase proteins (APPs) have been proposed as suitable veterinary biomarkers to monitor welfare, and inflammatory response. In addition, C-reactive protein (CRP) has recently been postulated as a potential biomarker use for vaccine safety studies and the measurement of haptoglobin (Hp) may be an indicator of average daily weight gain (ADWG) in pig farms. The aims of this study was to evaluate the response of Hp and CRP, the increase of temperature and the ADWG obtained after PCV2 and M. hyo vaccination with two vaccines mixture in Iberian piglets.
Materials and Methods: Two groups of 20 Iberian piglets were vaccinated, 7 days after weaning, with a single injection (2 mL) of (A) FLEXcombo®:Boehringer Ingelheim, Spain, SA) or with a single injection (2 mL) of (B) Porcilis® PCV-M Hyo (Intervet International B.V. Holland). Blood samples and weight of each animal were taken before vaccination (basal levels), 24h after vaccination (24h Post-V) and 48h after vaccination (48h Post-V). Also, the weight after 39 days of vaccination was taken (39d Post-V). The rectal temperature was recorded before and 8h after immunization. Serum Hp and CRP concentrations were determined using an automatic biochemical analyzer (Olympus 2700, Germany). A two-ways ANOVA test was performed and a value of $P<0.05$ was used to indicate significance.
Results: In the case of Hp concentrations with respect to baseline, significant differences in group B at 24h ($P<0.001$) and 48h ($P<0.01$) Post-V were found. CRP showed significant ($P<0.05$) higher values at 48h Post-V in group B compared with group A In relation to baseline, significant ($P<0.001$) increases in group B at 24h and 48h Post-V were observed, whereas in group A CRP increased only at 24h Post-V ($P<0.05$). 8h Post-V, rectal temperatures were significantly higher in the group B (41.0ºC) ($P<0.01$) compared to group A (39.9ºC). Group A showed significant higher values ($P<0.001$) in ADWF at 24h Post-V compared with group B (252 g vs. -72 g). In addition, the ADWG were higher in animals of group A compared with animals vaccinated at 48h Post-V (360 g vs. 238 g) and 39 days Post-V (344 g vs. 317) with a final average weight gain of 13 Kg for group A and 12 kg for group B.

Conclusion: According to the results obtained, the production of APPs has been higher and more persistent in animals of group B. In addition, this group had higher rectal temperature and less ADWG which are indicatives of greater inflammatory response and, therefore, a worse adaptation to weaning. This fact has also been observed with commercial piglets.

Disclosure of Interest: None Declared

Keywords: APPs, Iberian piglets, Vaccine

Vaccinology & Immunology

PO-PF3-195

Field efficacy of ERYSENG® for the control of a clinical swine erysipelas infection in a fattening unit.

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Introduction: The objective of this trial was to evaluate the efficacy of ERYSENG® in pigs by comparing the serological status against swine erysipelas, the appearance of clinical signs and productive parameters at the end of the fattening period, before and after vaccination.
Materials and Methods: The trial was conducted in a post-weaning/fattening facility located in France. This facility receives 270 twenty-eight days old piglets every 6 weeks from different farms.
Before vaccinating with ERYSENG®, numerous clinical cases of swine erysipelas were observed on the farm. The mortality rate was more than 10% in 2014. The cases of arthritis were numerous and required significant treatment. At the slaughterhouse, the rate of seizures for swine erysipelas was 1.12% (31 carcasses out of 2778). Serological analysis for swine erysipelas showed negative results for all 9-week-old piglets and positive results for 50% of 19-week-old piglets, showing a clear seroconversion during the fattening period. In terms of zootechnical values, the data for 2014 were as follow: an ADG of 645 g, FC of 2.84. The average duration of the fattening process was 169 days.
Taking into account this situation the decision was to start vaccinating all the piglets of the farm using ERYSENG®. The vaccine was administered at 10 weeks of age, intramuscularly with a 2 ml/dose. 10 serum samples were randomly taken from batch 1 (n=270) before vaccination, from batch 2 (n=270) 4 weeks after vaccination and from batch 3 (n=270) 14 weeks after vaccination. Antibodies to *E. rhusiopathiae* were titrated using a commercially available ELISA (CIVTEST® SUIS SE/MR); samples were considered positive when IRPC ≥ 40. Productive parameters were also monitored during the entire fattening period.
Results: Before implementing the vaccination with ERYSENG®, all the piglets were negative for erysipelas antibodies. 100% of the pigs from batch 2 and the batch 3 were 100% positive at 4 weeks and at 14 weeks after vaccination respectively.
On the farm, the symptoms of the disease disappeared. At the slaughterhouse, the seizure rate was 0.15%, that is an 89% decrease. For the first quarter of 2015, the ADG was 665g, that is a 20g improvement compared to 2014. The FC value was 2.72 for the first quarter of 2015, that is a 0.12 reduction, which gives an estimated profit of €2.76 per 100kg of carcass. Finally, the mortality rate during fattening was reduced to 6.8%. This reduction of 3.4% means a profit of €3.74 per 100kg of carcass.
Conclusion: Vaccination of pigs using ERYSENG®, induces effective protection against swine erysipelas, with regard not only to symptoms and mortality, but also to seizures at the slaughterhouse and productive parameters, such as the ADG and FCR.

Disclosure of Interest: None Declared

Keywords: Eryseng, Erysipelothrix rhusiopathiae, Swine erysipelas

Vaccinology & Immunology

PO-PF3-199

Secretory production of sugar ABC transporter substrate-binding protein from Mycoplasma hyopneumoniae in Bacillus subtilis

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Introduction: *Mycoplasma hyopneumoniae* is the principal aetiological agent of swine enzootic pneumonia, a chronic respiratory disease occurs worldwide. Control of *M. hyopneumoniae* infections can be accomplished by vaccination. Currently available *M. hyopneumoniae* vaccines are made from whole inactivated *M. hyopneumoniae* bacteria. However, production of these vaccines are complicated, expensive and time-consuming. Recently, considerable effort is being made to develop low cost easily-produced subunit vaccines. Our previous study showed that sugar ABC transporter substrate-binding protein (Mhp145) from *M. hyopneumoniae* is considered to be an attractive vaccine candidate. The gene encoding Mhp145 has been cloned and expressed in *Escherichia coli*. However, the purification of recombinant Mhp145 (rMhp145) from *E. coli* is time-consuming and labor-intensive. The objective of this study is to extracellularly express rMhp145 in *Bacillus subtilis* and establish a simple purification procedure for rMhp145.
Materials and Methods: Silent mutated Mhp145 gene which the nonsense TGA codons in the gene had been converted to TGG codons (tryptophan) was amplified by polymerase chain reaction from pET-Mhp145 and cloned into four secretion plasmids. The resulting plasmids, pSP1-Mhp145 to pSP4-Mhp145, were transformed into *B. subtilis* WB800N/pBL1, respectively. Transformants were incubated with shaking at 30°C to an OD_{600} of 0.4-0.5 and then induced with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 48 hours at 30°C. The supernatant samples of *B. subtilis* transformants were collected and analyzed by SDS-PAGE and western blot. The rMhp145 produced by the *B. subtilis* (pSP2-Mhp145) was purified from culture supernatant by immobilized metal-ion affinity chromatography (IMAC). Purity of rMhp145 was determined by SDS-PAGE.
Results: A 1.2-kb DNA fragment encoding mature Mhp145 with a C-terminal His-tag was cloned into four secretion plasmids. The resulting plasmids were transformed into *B. subtilis,* respectively. The expression of the rMhp145 by *B. subtilis* transformants was achieved by IPTG addition. Forty-eight hours after induction, a significant amount of rMhp145 were noticed in the supernatants of all transformants. The highest secretory expression level of rMhp145 was achieved when the Mhp145 fused to the SP2 signal peptide. The rMhp145 could be directly purified from the supernatant of *B. subtilis* by using IMAC. The final yield of rMHP145 was 18.31 mg/L with purity above 95%.
Conclusion: This study demonstrated the secretory production and purification of rMhp145 in *B. subtilis*. The purified rMhp145 will be further used in development of a subunit vaccine or cocktail vaccine.

Disclosure of Interest: None Declared

Keywords: ABC transporter substrate-binding protein, Bacillus subtilis, Mycoplasma hyopneumoniae

Poster Abstracts

Vaccinology & Immunology

PO-PF3-200

Efficacy against swine erysipelas conferred by ERYSENG® under field conditions.

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Introduction: The aim of this study was to demonstrate the efficacy of ERYSENG® against an *Erysipelothrix rhusiopathiae* (ER) infection in pigs under field conditions, by evaluation of the humoral immune response and the number of arthritis treatments during the entire fattening period.
Materials and Methods: Two hundred and forty 8-week-old Iberian pigs, from an 800-sow farm located in Salamanca (Spain), clinically healthy and free from antibodies against ER, were randomly assigned to a vaccinated group (n=120) or a control group (n=120). Animals from the vaccinated group were immunised three times i.m. with ERYSENG® (2 ml/dose, at 8, 12 and 23 weeks of age), a monovalent vaccine for the prevention of swine erysipelas adjuvanted with Hipramune® G^d. The control group remained unvaccinated until the end of the study. 30 serum samples/group were obtained on days 0, 27, 48, 82, 104, 138 and 173 and antibodies to ER were titrated using a commercially available ELISA (CIVTEST® SUIS SE/MR). The number of treatments was recorded from day 0 to the end of the trial. Percentages of arthritis treatments and the proportion of seroconversion against ER were compared between groups using the Chi-square test ($p<0.05$), whilst the serological response against ER was compared using the Mann-Whitney test ($p<0.05$).
Results: The vaccinated group received fewer arthritis treatments than the control group (37.90% versus 62% respectively). Vaccinated animals showed seroconversion after the primary vaccination (in weeks 8 and 12) and the booster dose (in week 23) with a significant increase in antibody titres after each immunisation. The mean ER-specific ELISA antibody titres in the vaccinated group exceeded the cut-off value (IRPC>40) and were statistically significantly different to the control group from day 27 until the end of the trial. On the other hand, the mean antibody titres from the control group remained below the cut-off (IRPC<40) throughout the trial. Regarding the percentage of seroconversion against ER, 96.4% of the animals from the vaccinated group were positive at the end of the trial (day 173), while a slight seroconversion was observed in the control group from day 48 onwards, and on day 173 nearly 15% of non-vaccinated animals seroconverted due to a field erysipelas infection.
Conclusion: Vaccination with ERYSENG® is a useful tool to reduce costs relating to arthritis treatments on farms with *E. rhusiopathiae* clinical problems. ERYSENG® provided protection from the first dose, with high antibody titres throughout the trial.

Disclosure of Interest: None Declared

Keywords: Eryseng, Erysipelothrix rhusiopathiae, Swine erysipelas

Vaccinology & Immunology

PO-PF3-201

Evaluate the efficacy of recombinant subunit vaccine against heterologous serotypes of Actinobacillus pluropenumoniae infection in swine

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Introduction: The necrotizing and hemorrhagic pleuropneumonia caused by *Actinobacillus pleuropneumoniae* (App) is a highly contagious and fatal respiratory disease of the pig. Vaccination is the best strategy for control of disease to diminish outbreak and economic loss in a contaminated pig farm. However, 15 different serotypes of App were recognized based on various capsular polysaccharides exhibited or displayed. Recently studies indicate that vaccination with App bacterins or containing varied capsular extracts could reduce mortality but protection against heterologous serotypes infection is not elucidated. The aim of this study was to evaluate the efficacy and cross-protection of Bayovac® ST-APP vaccine against heterologous App serotypes infection.
Materials and Methods: The specific-pathogen free (SPF) pigs were immunized with Bayovac® ST-APP vaccine twice at a three-week interval. All vaccine and placebo immunized pigs were endotracheally challenged with lethal dose of serotype 1 (App1), 2 (App2), or 7 (App7) *A. pleuropneumoniae* for cross-protection evaluation. The clinical signs, rectal temperature, mortality and subacute phase of lung lesion score at the 7 days after inoculation were evaluated.
Results: The Bayovac® ST-APP vaccine showed good safety and no inflammatory side effects were noticed in vaccine immunized pigs. No mortality and no typical fibrino-hemorrhagic lung lesions were observed after App1 and App2 inoculation in vaccinated group, and only 20% mortality with minor to moderate lung lesions accompanied with granulomatous changes were found using App7 challenge. However, 40% mortality with typical moderate to severe fibrino-hemorrhagic pleuropneumonia lesions were observed using App1 or App2 inoculation, and 80% mortality with massive and severe typical lung lesions using App7 challenge in placebo groups. In addition, the major clinical signs including depression, move slowly, vary dyspnea with rapid respiratory rate, coughing, and loss of appetite were noticed start at 3-4 hours immediately after challenge in all placebo immunized pigs. The lung lesion scores of vaccinated to placebo groups after challenge showed 13.0±18.5 to 56.4±43.3 (App1), 27.0±6.9 to 51.8±29.4 (App2), and 48.6±14.7 to 90.2±17.3 (App7), respectively.
Conclusion: The results indicated the Bayovac® ST-APP vaccine could elicit good cross-protective immunity against heterologous serotypes 1, 2, and 7 of *A. pleuropneumoniae* and reduce clinical signs, fever retention, mortality and lung lesions scores in SPF pigs. Additional animal trials are necessary to evaluate vaccine efficacy of cross-protection and its application in the contaminated pig farm.

Disclosure of Interest: None Declared

Keywords: Actinobacillus pluropenumoniae, Cross-protection, Heterologous serotypes

Vaccinology & Immunology

PO-PF3-204

Longitudinal Immune Response on PRRS in Vaccinated Pigs for 26 Weeks Period

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Introduction: The most economically important infectious disease in pig industry is Porcine Reproductive and Respiratory Syndrome (PRRS). Serological profiles of the PRRS using ELISA test is an important tool in monitoring and evaluating health status in farms**.** The dynamic of immune response on PRRS in a longitudinal fashion has not yet been studied in the real situation on farm in Thailand. The purpose of this study was to observe serological profiles on PRRS immune response, in pigs for 26 weeks period, in a longitudinal fashion.
Materials and Methods: A total of 126 blood samples were collected from 18 pigs in a self-replaced farm with history of PRRS-stabilized health status. The blood samples were longitudinally collected at 2, 6, 10, 14, 18, 22 and 26 weeks of age and assayed for ELISA using IDEXX PRRS X3 Ab Test (Idexx Laboratories, USA) as well as serum neutralizing (SN) titer. All pigs were vaccinated with MLV PRRS vaccine at 2 weeks of age.
Results: The result from ELISA test was presented as mean of sample to positive ratio (S/P) ± standard deviation. The overall average S/P was 1.432 ± 0.905 (0.014-3.192). The average of S/P in each age group was 1.315±0.73, 1.028±0.64, 1.339±1.08, 1.788±1.02, 1.621±1.00, 1.472±0.92 and 1.461±0.81, respectively. For the SN titer was $\log_2$ transformed and shown as mean ± standard deviation. The overall average of the SN titer was 2.302±0.906 (range 1.77 -3.55). The averages of SN titer at 2, 6, 10, 14, 18, 22 and 26 weeks were 3.556±0.784, 1.833±0.786, 1.722 ±0.575, 1.778±0.808, 1.778 ±0.878, 2.556±1.381, and 2.889±1.132, respectively. The lowest SN titer was 1.722±0.575 at 10 weeks, which occur with neutralizing of maternal derived antibody and MLV vaccine. The highest was 3.556 ±0.786 at 2weeks form maternal derived antibody.
Conclusion: This was a preliminary study observing ELISA titer and SN titer for PRRS in pigs in a longitudinal fashion. This would represent the status of protective immunity in pigs to PRRS. All samples in this study were collected from a stable herd, thus, the ELISA S/P showed low variability. However, the maximum S/P was found at 14 weeks of age, the highest in average S/P, and decline after 18 weeks of age. The study also showed that SN in pigs that had vaccinated once at 2 weeks of age could maintain SN titer for a long period of time, at least 24 weeks after vaccination, in this study. This might answer some questions asked about the preparation of replacement gilts on farm to acclimatize for the PRRS to keep stability of health status of sow herds on vaccination program.

Disclosure of Interest: None Declared

Keywords: ELISA, immune response, PRRS

Vaccinology & Immunology

PO-PF3-205

Comparative study of the effect of intradermal and intramuscular vaccination (UNISTRAIN® PRRS) on the course of seroconversion in PRRS-negative pigs

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Introduction: One of the key points in the control of PRRS virus (PRRSV) circulation on pig farms is the vaccination of piglets with a modified live vaccine (MLV). The MLV UNISTRAIN® PRRS applied via the IM route is an effective tool against the PRRSV. In the same way, this vaccine can now be applied via the ID route using a new needle-free injector device (Hipradermic®). The aim of this study was to compare humoral immune response following vaccination via the ID or the IM route in PRRS-negative pigs under field conditions.
Materials and Methods: Two PRRS-negative fattening farms, housing around 430 pigs (Farm A) and 360 pigs (Farm B), 10-weeks old, were randomly divided into two different groups, the IM group and the ID group. Thirty-five and 42 healthy animals from Farm A and Farm B, respectively, were randomly selected to both the IM and ID groups and individually marked. In the same way, 12 pens on Farm A and Farm B, respectively, were selected for each group. The IM group was vaccinated via the intramuscular route with UNISTRAIN® PRRS (2 ml/dose) and the ID group was vaccinated via the intradermal route with the same vaccine (0.2 ml/dose) using Hipradermic®.
The two groups were compared by analyzing the PRRSV antibody response by ELISA and PRRSV detection by RT-PCR in serum samples from individual animals and oral fluid from pens prior to vaccination and at 28 and 48 days post-vaccination (dpv). In addition, the local reaction and vaccine losses were evaluated in individual animals in the ID group. Different statistical tests were performed according to the recorded data.
Results: Prior to vaccination, all the samples were negative for RNA and PRRSV antibodies. After vaccination, the PRRSV antibody response in both groups showed a significant increase at 28 and 48 dpv (Spearman; $p<0.05$). Moreover, these antibody levels for all days were similar for the ID and IM groups on Farm A. However, the antibody levels at 48 dpv were significantly lower in the IM group than in the ID group on Farm B at individual and pen level (Mann Whitney; $p<0.05$). With regard to safety, vaccine losses were not detected at the time of inoculation, whilst 4 hours later, animals showed 0.04 % mild inflammation and 10.4% crust, but these were transient, resolving within 2 days.
Conclusion: Vaccination with UNISTRAIN® PRRS induces humoral response in PRRS-naïve pigs regardless of the injection technique used. Both injection techniques had a comparable effect on the antibody response at 28 and 48 dpv, although some higher levels were observed with the ID route. Vaccination with UNISTRAIN® PRRS ID seems to be a new, safe and immunogenic method for PRRS control plans.

Disclosure of Interest: None Declared

Keywords: intradermal vaccination, PRRS MLV vaccine

Poster Abstracts

Vaccinology & Immunology

PO-PF3-207

Circumvent® PCV M G2: Field production data update

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Introduction: Previously, we reported on the production record analysis of a swine operation where weaned pigs are purchased from two sow farms and are kept in separate flows. One flow consists of terminal line animals that are sold for slaughter (TERM). The other flow consists of maternal line animals where most of the gilts are sold as replacements (MAT). Both flows are of high health status. The previous reports addressed differences in production performance between the flows, which over time used different PCV2 vaccination programs. Starting in mid-2013, both flows used Circumvent® PCV (PCV-G1) and in early 2014, both flows were switched to Circumvent® PCV G2 (PCV-G2). The objective of the production data analysis reported here was to compare the performance of PCV-G1 to PCV-G2 within each flow.
Materials and Methods: Vaccination with either Circumvent product was done per label at 3 and 6 weeks of age (WOA) using 2 mL for each injection with PCV-G1 and 1 mL for each injection at 3 and 6 WOA with PCV-G2. Production data was collected by site as each group was closed-out. Only finisher data was evaluated as nursery performance was the same in both flows. The data was analyzed on a close-out group basis or an individual pig basis. For the close-out based analyses, the group average daily gain (ADG),feed conversion ratio (FCR), mortality rate and culled rate were tested by ANOVA to detect significant differences between the vaccines (PCV-G1 vs. PCV-G2) within each flow. Analyses based on the individual pig (mortality and culled) were performed by Chi Square.
Results: The number of groups (number of pigs) analyzed by flow and vaccine was: 33 (68,074) for TERM-G1; 60 (131,463), TERM-G2; 25 (71,371), MAT-G1; and 37 (103,819), MAT-G2. Production parameters by flow and vaccine (G1 vs. G2) were: mortality rate- TERM, 2.32% vs. 1.58% and MAT, 1.77% vs, 1.54%; cull rate- TERM, 1.14% and 1.03% and MAT, 0.68% vs. 0.37%; daily gain (lb)- TERM, 2.028 vs. 2.072 and MAT,1.956 vs. 1.941; and feed conversion ratio- TERM, 2.664 vs. 2.612 and MAT, 2.682 vs.2.622. For daily gain and feed conversion ratio, there were no differences between vaccines within each flow. In the terminal flow, mortality rate was significantly lower in the PCV-G2 vaccinated pigs. In the maternal flow, the mortality and cull rates were significantly lower in the PCV-G2 vaccinated groups. However, these differences were relatively small and became statistically significant due to the large sample size.
Conclusion: Overall, the performance of PCV-G2 appears to be at least equal to, if not slightly better than, PCV-G1.

Disclosure of Interest: B. Thacker Conflict with: Merck Animal Health, J. Creel Conflict with: Merck Animal Health, R. Blomme: None Declared

Keywords: growth performance, PCV2 vaccine

Vaccinology & Immunology

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PCV2 Antibody Elisa Titer in Group of Pigs vaccinated with PROVAC® CIRCOMASTER under an Off-Label Program in field conditions.

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Introduction: Vaccination against PCV2 has become widely accepted by majority of commercial farms. Commercially available vaccines offer program and positioning with difference with each other. PROVAC® CIRCOMASTER (Komipharm International Ltd., KOREA) is the first vaccine with approved program for both sows & piglets. In piglets, it has a two-doses positioning to assure the fattening pigs are protected longer up to the period they are slaughtered. Clinical trial using PROVAC CIRCOMASTER in the Two-dose program results to lower post-weaning mortality rate, higher weight at four months old and higher increase in SP value compared with control group.
The aim of the study is to determine the serological performance in pigs vaccinated with PROVAC® CIRCOMASTER following a modified (Off-Label) program in Piglets given with just one-dose program at 3 weeks of age.
Materials and Methods: The farm selected in this study is a 400 sow multiplier farm located at region 4A in Luzon Island in The Philippines. The farm is using PROVAC® CIRCOMASTER in the past 2-3 years in both breeders and piglets. The Breeders received mass vaccination every 4 months and the young pigs are vaccinated at 3 weeks of age with just one-dose (1 ml) of PROVAC® CIRCOMASTER. No booster dose was given. A cross-sectional blood sampling was performed on December 21, 2015 collecting five serum samples from each different age as follows: 4 weeks old, 8 weeks old, 12 weeks old, 16 weeks old, 20 weeks old & 24 weeks old. The sample was submitted to a laboratory diagnostic lab (Bioassets Diagnostic Laboratory, Batangas, Philippines) and test for PCV2 Ab ELISA test (BIOCHEK®).
Results: PCV2 Elisa Antibody results are as follows:
The 4 week's old Mean S/P ratio is 2.17 with Standard Deviation of 0.36. The 8 weeks old Mean S/P ratio is 1.32 with Standard Deviation of 0.35. The 12 weeks old Mean S/P ratio is 2.69 with Standard Deviation of 1.25. The 16 weeks old Mean S/P ratio is 3.85 with Standard Deviation of 1.24. The 20 weeks old Mean S/P ratio is 3.37 with Standard Deviation of 0.49. The 24 weeks old Mean S/P ratio is 2.30 with Standard Deviation of 0.55. The above serological results reflected a homogenous and protective Antibody level against PCV2 virus. It also reflected that a single vaccination of PROVAC® CIRCOMASTER at 3 weeks old have maintained a protective Antibody titer level extended possibly even beyond 24 weeks old.
Conclusion: It can be concluded that PROVAC® CIRCOMASTER even at an OFF-Label program can induce duration of immunity (DOI) of more than 21 weeks.

Disclosure of Interest: None Declared

Keywords: Off-Label, PROVAC CIRCOMASTER

Vaccinology & Immunology

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The protection efficacy of the enterotoxigenic Escherichia coli vaccine candidate by GI24 against neonatal piglet colibacillosis

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Introduction: Enterotoxigenic *Escherichia coli* (ETEC) causes diarrheal disease and is the most common enteric colibacillosis encountered in neonatal piglets, and is considered as one of the most economically important diseases in the pig industry worldwide. ETEC uses extracellular fimbriae to adhere to the surface of intestinal epithelia. After adhesion, ETEC release enterotoxins, namely, heat stable enterotoxin and heat-labile enterotoxin, which induce fluid excretion and cause severe diarrheal disease in the neonatal piglets. Therefore, adhesive fimbriae play critical role in ETEC colibacillosis. In this study, F4+, F5+, F6+ and F41+ ETEC strains were lysed by GI24. Subsequently, the effectiveness of the lysed cells as a vaccine candidate against piglet colibacillosis was evaluated in piglet.
Materials and Methods: GI24 (GRFRRLRKKTRKRLKKIGKVLKWI-NH_2) peptides were chemically synthesized. The peptide was used to lyse the vaccine strains. All group sows (n=6) were intramuscularly (im) immunized by administration of two doses of vaccine, at 11 weeks of pregnancy and at 14 weeks of pregnancy. Group A sows were immunized with 1 ml of sterile PBS. Group B sow were immunized with a total of 2×10^9 the mixture of ETEC vaccine candidates (the mixture containing 5×10^8 cells of each ETEC lysate cells) in 1ml PBS. Blood samples were taken at 0 (prior to prime immunization), 3 (prior to the second immunization) and 6 (on the day of farrowing) weeks post prime immunization (WPPI). Colostrum samples were collected from the sows on the day of farrowing. All piglets were orally challenged with 3 ml of the mixture containing 1×10^9 CFU/ml of each challenge strain at 5-day-old. All piglets were monitored daily for diarrhea and mortality for 14 days after challenge.
Results: Serum IgG titers against each F4+, F5+, F6+ and F41+ ETEC whole cell antigen were significantly increased in all immunized sows compared to those in group A at 3 and 6 WPPI ($p\leq0.05$). In immunized group sows, colostrum IgA and IgG concentrations against all ETEC whole cell antigens were significantly increased compared to those of unimmunized group sows on the day of farrowing. The serum IgG and IgA titers against all ETEC strain whole cell antigens in group A piglets were significantly higher than those of control piglets on day 4 after birth. Group B piglets did not exhibit clinical signs such as diarrhea up to day 14 after challenge. In contrast, diarrhea was observed in 15 of 23 group A piglets (65.2%) and 4 died due to severe diarrhea.
Conclusion: These findings indicate that intramuscular immunization of sows with the mixture of the ghost cells can effectively protect their offspring from colibacillosis.

Disclosure of Interest: None Declared

Keywords: enterotoxigenic Escherichia coli, Ghost Vaccine, Neonatal diarrhoea

Vaccinology & Immunology

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Heterogeneous expression of Toll-like receptors 1-10 genes in lymphoid tissues of different ages pigs

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Introduction: Age-associated variation of immune responsiveness is well-known in pigs. Toll-like receptors (TLRs), as pathogen recognition receptors, recognize the microbial components. The details expression kinetics of TLRs may help to understand the immune responsiveness of pigs, but the expression patterns of all TLRs have not yet been studied in pigs. Therefore, the aim was to study the expression pattern of the TLR family (TLR1-10) in different lymphoid tissues collected from pigs at different ages.
Materials and Methods: Nine clinically healthy female Pietrain pigs of three age groups newborn (one day), young (2 months), and adult (5 months) were selected considering the commercial pig farming. Each age group consisted of three animals. All pigs were kept in the same environmental, sanitation and housing condition. After slaughter, tissues from the cervical lymph nodes (CLN), liver, spleen, thymus, lung, heart and skin from the ear were collected. PBMC was isolated from whole blood using Ficoll-Histopaque. Total RNA from each samples was isolated (Tri-reagent) including DNA clean-up, and the concentration was quantified (Nanodrop). TLR (1-10) transcripts were quantified in GenomeLab™ GeXP Genetic Analysis System (Beckman Coulter). Geometric mean of housekeeping genes (*ACTB*, *GAPDH* & *TBP*) was used for normalisation. The average of biological repeats was considered for further analysis (SAS ver9.2).
Results: mRNA expression showed that TLR1 was highly expressed in the CLN and spleen, and moderately in the liver and lungs; whereas TLR2 expression was higher in the liver, lung and spleen. TLR3 mRNA was the most abundant in all tissues and higher expressed in thymus, kidney, lungs and liver. In the spleen, all TLRs (except TLR5) expressions were higher ($P < 0.01$) in young pigs compared to newborn piglets. In the thymus, TLR3 expression was significantly higher in young pigs compared to newborn and adult. The Western blot results of TLR2, 3 and 9 in selected tissues (CLN, spleen and lung) appeared to be consistent with the mRNA expression results. Cells in lungs, spleen and CLN were positively immunostained for TLR2, 3 and 9. Variance analysis showed that both age and organ types have an effect on all TLRs expressions ($P < 0.001$).
Conclusion: This study sheds light on the expression patterns of TLR (1-10) genes in important lymphoid tissues in pigs of different ages. The heterogeneous expression of TLRs with ages may contribute to the altered immune responsiveness of pigs with ages.

Disclosure of Interest: None Declared

Keywords: innate immunity, mRNA, protein

Poster Abstracts

Vaccinology & Immunology

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Efficacy of a multivalent vaccine in the prevention of porcine respiratory disease complex

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Introduction: Porcine Respiratory Disease Complex (PRDC) is a multifactorial syndrome which is a source of heavy economical losses. Its control implies herd management and therapeutic measures (vaccines, antimicrobials). Objective of this study was to assess efficacy of a multivalent vaccine to prevent PRDC and related losses in a Philippine herd.
Materials and Methods: The study was performed in a farrow-to-finish herd with1,400 sows having a history of respiratory diseases (isolation of *Pasteurella multocida, Bordetella bronchiseptica* and *Streptococcus suis* from lung samples). Twenty sows were randomly allocated to a Tested (T) or Control (C) group according to parity. Sows in the C group were not vaccinated whereas sows in the T group received a multivalent inactivated vaccine twice, respectively 5 and 3 weeks pre-farrowing. The tested vaccine comprised bacterins and toxoids of *Pasteurella multocida, Bordetella bronchiseptica, Actinobacillus pleuropneumoniae* (App) and bacterins of *Streptococcus suis*, *Haemophilus parasuis* (Hps) *and Mycoplasma hyopneumoniae* (Mhyo), (Suigen® PRDC, Virbac). All piglets born from vaccinated sows received the same tested vaccine at 3 and 5 weeks of age instead of the routine respiratory vaccine program (against App, Hps and Mhyo) which was applied to piglets from control sows. The tested vaccine was injected intramuscularly at the dose of 2 ml per sow and 1.5 ml per piglet. Pigs were housed in collective pens per group from weaning to slaughtering during which respiratory cases were recorded according to a standardized clinical examination. Mortality and morbidity rates due to respiratory cases were compared between groups by the Fisher's exact test. The Return On Investment (ROI) per vaccinated piglet was calculated as the ratio between earning and vaccination cost, earning being the mean difference of profit between groups (price of pig weight produced minus feed, vaccine and antibiotic treatment costs).
Results: Respectively 94 and 96 piglets were included in T and C groups. No side effects were observed after vaccination of sows or piglets. Mortality and morbidity rates from weaning to slaughtering were significantly lower in T group than in C group (respectively 10.8% and 24.4% for mortality and 21.7% and 38.9% for morbidity, $p<0.05$). The ROI of the tested vaccine per piglet was equal to 4.2.
Conclusion: Reduced incidence of respiratory cases from weaning to slaughtering may be due to passive immunity acquired from vaccinated sows followed by active immunization of vaccinated piglets. As a consequence less antibiotic treatments were necessary in T group and higher total pig weight was produced, reflected in a positive ROI of the vaccine program tested.

Disclosure of Interest: None Declared

Keywords: bacteria, respiratory disease, Vaccine

Vaccinology & Immunology

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Secretory expression of 46-kilodalton surface antigen from Mycoplasma hyopneumoniae in Bacillus subtilis

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Introduction: *Mycoplasma hyopneumoniae* is the causative agent of swine enzootic pneumonia (SEP), one of the most important chronic respiratory diseases that affects swine production worldwide. Vaccination is the most effective method for control of SEP. A plethora of studies indicated that 46 kDa surface antigen (P46), a highly conserved and immunodominant antigen, is considered to be a potential vaccine candidate. Our previous study has cloned and expressed recombinant P46 (rP46) in *Escherichia coli*. However, the purification of rP46 from *E. coli* is time-consuming. In comparison to *E. coli*, *Bacillus subtilis* is a more attractive host because it has the capacity to secrete proteins into the growth medium, which significantly simplify the downstream processing. Therefore, the aim of this study is to use *B. subtilis* as a host for secretory expression and one-step purification of rP46.
Materials and Methods: Silent mutated P46 gene which the nonsense TGA codons in the gene had been converted to TGG codons (tryptophan) was amplified by PCR from pET-P46 and cloned into five different secretion plasmids. The resulting plasmids were transformed into *B. subtilis* WB800N/pBL1 using electroporation, respectively. Transformants were incubated with shaking at 30°C to OD_{600}=0.4-0.5 followed by induction with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 48 hours at 30°C. Supernatant samples of *B. subtilis* transformants were collected and analyzed by SDS-PAGE and western blot. Recombinant P46 (rP46) produced by *B. subtilis* (pSP2-P46) was purified from culture supernatant by immobilized metal-ion affinity chromatography (IMAC). Purity of recombinant P46 was determined by SDS-PAGE.
Results: A 1.2-kb DNA fragment encoding mature P46 with a C-terminal His-tag was amplified by PCR and cloned into five secretion plasmids. The resulting plasmids, pSP1-P46 to pSP5-P46, were transformed into *B. subtilis* and used for expression of the rP46 protein. The expression of the rP46 by *B. subtilis* transformants were achieved by addition of IPTG into the culture medium. Forty-eight hours after induction with IPTG, secretory expression of rP46 were observed for all transformants. The secretory expression of rP46 reached the highest level when P46 fused to the SP2 signal peptide. The rP46 could be directly purified from the supernatant of *B. subtilis* (pSP2-P46) by using IMAC with a yield of 22.55 mg/L and a purity of >95%.
Conclusion: This study presents the high-level secretory expression and one-step purification of rP46 in *B. subtilis*. The purified rP46 will be further used in subunit vaccine and enzyme-linked immunosorbent assay (ELISA) kit developments.

Disclosure of Interest: None Declared

Keywords: 46-kilodalton surface antigen, Bacillus subtilis, Mycoplasma hyopneumoniae

Vaccinology & Immunology

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The impact of discontinuing PRRS MLV vaccination in piglets in the single site farm in Thailand.

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Introduction: Modified live virus (MLV) vaccines are widely used in PRRS control strategies, have been shown to reduce clinical signs and production losses. The VR2332-based vaccine is the most widely considered method to control the fatal disease in Thai swine farms. As a results of herd stabilization by the VR2332 based virus vaccine, the productivity has improved after implementing the control strategies.
During stable pig performance, some farms have planned to expand their pig business. In order to reduce production cost, farmers had tried to stop vaccination against PRRSv in piglets. The aim of this study was to investigate the effect of stopping PRRS MLV vaccination in the nursery unit in a high pig density area in Thailand.
Materials and Methods: The farm is a farrow to finish farm with 1,700 sows in the western area of Thailand. Farm is positive for PRRS, MH, PCV2, PED, CSF, APP and PRV. Farm has been stabilized sows herd with VR2332 based virus vaccine for 8 years.
In the farrowing unit, piglets were vaccinated against PRRSv at 2 weeks, CSF at 3 weeks and PCV2-MH at 4 weeks. The weaning age is 26-28 days. The nursery losses observation was done during batch No.1 to batch No.22 year 2015. Batch No.11 – Batch No.22 are stopping piglets vaccination by PRRS MLV vaccine period. Due to high losses in nursery, farmers decided turn to piglets vaccination again since Aug 2015 until up to date (data not show). A total 13,038 pigs were evaluated in this study. There are 6,130 of the piglets in batch no. 1-10 were vaccinated with VR2332 based virus vaccine (MLV group) and 6,908 pigs of batch no.11-22 were not vaccinate (NVC group). To evaluate the impact of stopping vaccination against PRRSv, a Total losses; Mortality, Culling rate were compared between the groups by Chi Square test, OpenEpi.Version3.
Results: The average total losses in nursery between MLV group and NVC group are 5.51% and 22.70% respectively. There was a statistic significant difference with regard to total losses ($p<0.001$). The average weight-out of MLV group is 29.9 kg and 28.81 kg of the NVC group. The cost was calculated automatically by the computer program. The benefit-to-cost ratio between non-vaccinated (NVC) and vaccinated (MLV) is 7.03:1 based on the mortality alone.
Conclusion: Based on the mortality change, stopping PRRS MLV vaccination resulted in big economic loss in this farm. Stopping PRRS MLV vaccination especially in single site farm, can be elucidated, results to higher PRRSv load and can be shed to the other production sites.

Disclosure of Interest: None Declared

Keywords: Economic, Nursery losses, Vaccination

Vaccinology & Immunology

PO-PF3-222

Vaccination Compliance: Are standard delivery doses non-standard doses?

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Introduction: Modern pig farming requires to set up adequate vaccination programs to prevent or decrease economic loss from important infectious diseases. Vaccination compliance is a key success factor for a cost-effective prevention strategy. It relies on the administration of the correct dosage, by the correct route at the correct date. During the vaccination process, leakage or bleeding out of vaccine droplets may be observed on the pig skin surface, particularly when using conventional vaccination material. Even when obtaining the expected efficacy results, the observation by the operator of this may affect the psychological sensation that pigs are appropriately vaccinated. The objective of this study was to evaluate the quantity of vaccine on the skin of pigs following a standard intramuscular injection in the neck.
Materials and Methods: Sixteen piglets, 4 per weight category i.e. 4-5 kg, 6-7 kg, 8-10 kg and 15-20 kg, were randomly selected in a commercial farm. No vaccine had been injected in the neck before the study day. Following cleaning and drying of the skin surface, 0.5-mL of CIRCOVAC® (Merial, Lyon, France), was injected, IM in the neck, according to the manufacturer instructions. Before injection a piece of blotting paper (4cm², 90g/m²) in an empty screw cap tube was weighed using a qualified precision scale. Just after injection, the piece of paper was applied on the skin surface at the injection site for 2 seconds then immediately stored in the screw cap container and the tube was then weighed. The quantity of vaccine lost on the skin surface was calculated by difference. The visible collection of any trace of blood, another other body fluid or exogenous water on the blotting paper led to the exclusion of the observation.
Results: On average, the volume of vaccine lost on the skin was 1.28% ± 0.85% of the standard dose (n=14), ranging from 0.20% to 3.45%. The average percentage per dose remaining on the skin surface were 1.67%, 1.27%, 0.88% and 1.15% for pigs vaccinated with 0.5 mL of CIRCOVAC and weighing 4-5 kg, 6-7 kg, 8-10 kg and 15-20 kg respectively, indicating the absence of age-dependency for the observation of this phenomenon.
Conclusion: Under the conditions of the study, the volume of a vaccine present on the skin of a pig following a standard intramuscular injection in the neck was 1.28% of the administered dose, what was considered as acceptable. Vaccination compliance was thought not to be impacted as vaccines doses are planned to accommodate this variation in delivery dose.

Disclosure of Interest: S. Paranese: None Declared, G. Leotti Conflict with: Merial Italia SpA, G. Sarli: None Declared

Keywords: Compliance, Vaccination

Poster Abstracts

Vaccinology & Immunology

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The use of a modified live PED vaccine in the Philippines

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Introduction: Porcine Epidemic Diarrhea (PED) is a Coronavirus infecting pigs globally but most especially in Asia1,2,3. The outbreaks have been causing problems in Philippine commercial farms. This field trial was conducted to confirm the efficacy of a Modified Live PED Vaccine (Enterisol® PED) in a commercial farm under field conditions.
Materials and Methods: The trial was conducted in a 500-sow farrow to finish farm in the North part of the Philippines, in a pig-dense area. The farm had historical PED-like breaks in 2006 and then again in 2008. Despite vaccination, the farm was still having poor performance especially high mortalities (32%) per month on a 12-month average) and low weaning weights.
After being confirmed positive for PED by PCR in January 2013, the farm decided to evaluate a modified live PED vaccine (Enterisol PED MLV) in August 2013 given parenterally, twice at 6 weeks and then again at 2 weeks pre-farrow in sows and gilts.
Using the chi-square and using Student's t-test (SAS 9.4.). the results of the vaccinates were compared with historical batch performances. Both local and systemic reactions were observed. The pre-weaning mortality and weaning weight results were compared. Results were considered significant, if $p \leq 0.05$.

Results: There was neither local nor systemic reaction observed in the vaccinated group following the two immunizations. A total of 86 sows (42 controls, 46 vaccinates) with a total of 968 piglets (450 controls, 518 vaccinates) were included into the trial. Vaccination yielded a significantly lower pre-weaning mortality (30% for control and 18% for vaccinates).
Mean weaning weight of the pigs from vaccinated sows were 66.31 kg versus 49.49 kg in the non-vaccinated controls.

Conclusion: The modified live vaccine used in this study proved to be efficacious in reducing the clinical impact of a PED infection. Vaccination of sows is beneficial with regard to pre-weaning mortality and weaning weight of the litter. Successful control of PED needs to include appropriate biosecurity measures, cleaning and disinfection and movement restrictions. Further studies are under way to confirm the efficacy of Enterisol PED MLV under different conditions. Meanwhile, PED remains a challenge for the SE Asian pig industry.

Disclosure of Interest: None Declared

Keywords: Coronavirus, Modified, Vaccine

Vaccinology & Immunology

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Effect of Aujeszky's disease pig vaccination on lung lesions of slaughtered pigs

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Introduction: Aujeszky's disease (AD) virus causes nervous disorders and high mortality rates in young animals and respiratory illness in older pigs. The main aim of this study was an estimation of the role of AD virus in PRDC in finishing pigs and the influence of pig vaccination against AD on lung protection.

Materials and Methods: The study was conducted in a Russian large farrow-to-finish production system (17,000 sows) where all four farms of the system are exposed to PCV2, EU PRRSV, *M.hyo*, AD (gE) and *App*. All pigs in the system included in this trial were vaccinated against PCV2, *M.hyo* and *App* with FLEXcombo® and Ingelvac APPX®. Scheme of AD vaccination: gilts twice before entering in a reproductive herd, sows and boars – quarterly with marker vaccine Ingelvac® Aujeszky MLV. Pigs in treatment groups (1050 pigs in site A and 1993 pigs in site B) were vaccinated intramuscularly at 62 and 77 days of age with the same vaccine. Control groups (3374 pigs in site A and 2715 pigs in site B) were not vaccinated against AD. Pigs of both groups were kept under same management conditions in neighboring rooms. Lungs of slaughtered pigs were estimated according to PigMon protocol (Straw et al., 1986). The Chi-square test was applied to analyze the results.
Results: *Site A.* Control groups (200 lungs): Lungs with pneumonia 10%, Lung with pleurisy and abscesses 50%, Average % of affected pulmonary parietal surface 1.16%, Average % of affected pulmonary parietal surface with active pneumonia 11.55%, Lungs with pulmonary parietal surface lesions ≥5% 5.5%.
Vaccinated AD groups (300 lungs): Lungs with pneumonia 3%, Lung with pleurisy and abscesses 23.7%, Average % of affected surface out of all lungs 0.15%, Average % of affected pulmonary parietal surface with active pneumonia 5.11%, Lungs with pulmonary parietal lesions ≥5% 1.3%.
Site B. Control groups (200 lungs): Lungs with pneumonia 28%, Lung with pleurisy and abscesses 35%, Average % of affected pulmonary parietal surface 4.45%, Average % of affected pulmonary parietal surface with active pneumonia 15.88%, Lungs with pulmonary parietal surface lesions ≥5% 23%.
Vaccinated AD groups (900 lungs): Lungs with pneumonia 18.1%, Lung with pleurisy and abscesses 14%, Average % of affected pulmonary parietal surface 1.92%, Average % of affected Pulmonary parietal surface with active pneumonia 10.63%, Lungs with Pulmonary parietal surface lesions ≥5% 13.9%.
Conclusion: In both fattening sites AD vaccinated pigs showed a statistically significant lower severity of pneumonia, pleurisy and lungs with lesions ≥5% of parietal surface of lung. These results confirm the active participation of AD virus in PRDC in fattening pigs.

Disclosure of Interest: None Declared

Keywords: Aujeszky's disease, Ingelvac Aujeszky MLV, lung check

Vaccinology & Immunology

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Field safety and efficacy study of a Mycoplasma hyopneumoniae and PCV2 combination vaccine in a pig herd with late onset of M. hyo infection

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Introduction: *Mycoplasma hyopneumoniae* (M.hyo) and Porcine Circovirus type 2 (PCV2) are two major infectious agents among others which are implicated in the Porcine Respiratory Disease Complex (PRDC). Vaccines against PCV2 and M.hyo are routinely used in the pig industry. The application of a combined vaccine against both pathogens leads to a reduction of the number of injections thus reducing handling stress for the piglets and labor for the user. The objective of the present study was to assess the safety and efficacy of a ready-to-use vaccine against M.hyo and PCV2 in a farm with a late M.hyo infection in the finishing unit. The farm was previously only vaccinating against PCV2.
Materials and Methods: A field study was done according to a randomized and blinded design in a farrow-to-finish farm in Germany. A total of 525 healthy suckling piglets of 21 (+3) days of age were allocated according to sex and weight to three treatment groups: V1 (n=175, 2 ml Porcilis® PCV M Hyo), V2 (n=175, 2ml combined M. hyo, PCV2 commercial vaccine) and control group CG (n=175, 2 ml sterile buffered saline). Twenty five (25) animals of each study group were included in the safety group for safety assessments and blood samples. Clinical observations and local injection site reactions (ISR) were checked 30 to 60 min after vaccination and daily for the next seven days. Rectal temperature of the safety animals was measured daily for four days. All study animals were weighed individually at admission (T0), after seven weeks (T49) and at the end of finishing one day before header pigs were sent to slaughter (T133). The lungs were examined at slaughter to score the severity of lung lesions according to Goodwin and Whittlestone 1973.
Results: No systemic side effects were observed in any of the vaccinated pigs. ISRs were observed in all study groups with a maximum diameter of 0.5cm in V1, 1cm in V2 and 0.5cm in CG. In all treatment groups, mean rectal temperatures remained within physiological range over the observation period. The mean average daily weight gain (ADWG) from T49-T133 was 894 g (V1), 896 g (V2) and 887 g (CG). Although V1 vaccinated animals had numerically higher ADWGs in the fattening period than CG animals, these differences were not statistically significant. The mean lung lesion score in V1 group (5.8) was significantly lower compared to CG (8.2) (p=0.003).The mean lung lesion score in V1 was 34.1 % lower than in V2 (8.8) (p=0.02).
Conclusion: Vaccination of piglets at 21 (+3) days of age with Porcilis® PCV M Hyo is safe and efficacious in reducing the severity of lung lesions under field conditions with a late infection of M.hyo in the finishing unit.

Disclosure of Interest: None Declared

Keywords: Combined Vaccine, Mycoplasma hyopneumoniae, PCV2

Vaccinology & Immunology

PO-PF3-228

Influence of Aujeszky's disease vaccination on main finishing parameters in large farrow-to-finish production system

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Introduction: Aujeszky's disease (AD) is a highly contagious, economically significant disease of pigs. Vaccination against AD with marker vaccines is an important part of eradication programs in endemic areas. The main aim of this study was an estimation of benefits of growing pig vaccination against AD.
Materials and Methods: The study was conducted in a Russian large farrow-to-finish production system (17,000 sows). All four farms of the system are seropositive to PCV2, EU PRRSV, *Mycoplasma hyopneumoniae* (M.hyo), AD (gE) and *Actinobacillus pleuropneumoniae* (APP). The breeding herd of both farms was vaccinated against EU PRRS, AD and APP. All pigs in the system included in this trial were vaccinated against PCV2 and M.hyo with FLEXcombo® and against APP with Ingelvac APPX® as a routine. Scheme of AD vaccination: gilts twice before entering in a reproductive herd, sows and boars – quarterly with marker (gE-negative) vaccine Ingelvac Aujeszky MLV. Pigs in treatment groups (3043 pigs) were vaccinated intramuscularly at 62 and 77 days of age with the same vaccine. Control groups (6089 pigs) were not vaccinated against AD. Control and vaccinated groups were kept under same management conditions in neighboring rooms of the same barn. The Chi-square test was applied to analyze the results.
Results: *Fattening site A.* Control groups (3374 pigs): mortality 2.73%, cull 1.51%, total losses 4.24%, ADG 791±24 g, final age 171±4 days and final live weight 104.88±2.04 kg.
Vaccinated AD groups (1050 pigs): mortality 0.86%, cull 1.90%, total losses 2.76%, ADG 764±13 g, final age 172 days and final live weight 101.19±0.26 kg.
All sera of both groups were seronegative to gE at 120 days old, but at 160 days control pigs demonstrated 100% positive results, vaccinated pigs kept gE-seronegative status.
Fattening site B. Control groups (2715 pigs): mortality 3.17%, cull 1.33%, total losses 4.49%, ADG 753±29 g, final age 176±5 days and final live weight 105.19±2.18 kg.
Vaccinated AD groups (1993 pigs): mortality 2.36%, cull 1.25%, total losses 3.61%, ADG 786±13 g, final age 170±4 days and final live weight 104.75±0.24 kg.
Conclusion: Vaccinated pigs of fattening site A demonstrated significant differences (X2>3.84) for mortality and total losses compared non-vaccinated control groups (0.86% and 2.76% vs. 2.73% and 4.24%).
There were not significant differences for mortality, cull, total losses on fattening site B. But AD vaccinated pigs demonstrated higher ADG (+33 g) and mean slaughter age decreased by 2.31 days than control. This field study show direct benefits of vaccination against Aujeszky's disease in positive herds a significant improvement in performance of fattening.

Disclosure of Interest: None Declared

Keywords: Aujeszky's disease, Ingelvac Aujeszky MLV

Poster Abstracts

Vaccinology & Immunology

PO-PF3-236

FIELD STUDY COMPARING TWO VACCINES AGAINST ATROPHIC RHINITIS IN BRAZIL

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Introduction: Atophic Rhinitis (AR) can lead to reduced daily gain, poor body condition and variable growth, due to difficulty in eating. It may also increase the risk of infection by respiratory diseases (1). Vaccination of sows against AR aims to protect piglets by passive immunity transmitted via the colostrum. The prevalence and intensity of AR is often assessed by scoring nasal lesions (2).
The main objective of this study was to evaluate the two years evolution of the Atrophic Rhinitis turbinate nasal lesion score and the prevalence of turbinate lesion at slaughterhouse, using different AR vaccines in every year.
Materials and Methods: The evolution study was carried out in different fattening pig batches coming from a 5000 sows farm from Paraná state in Brazil. The farms were historically vaccinating against AR with a vaccine with an adjuvant based on dl-α-tocoferol (Vaccine A), the vaccination program used was two doses to the gilts, eight and four weeks before farrowing and one dose to the sows four weeks prior farrowing. At the beginning of the study were examined 35 nasal turbinates at slaughter (10% expected prevalence, 95% confidence level). Nasal turbinates were randomly selected from pigs coming from vaccinated sows. The nasal lesion score was performed throughout the method developed by Embrapa-CNPSA, this method consists in four degrees of turbinate lesion. From the turbinate lesion score the prevalence and the index of nasal lesion is calculated (3,4). After this first evaluation, the farm technical team decided to change the vaccine used in the farm to another vaccine: RHINISENG®. The vaccination program with RHINISENG® was the same used with the previous vaccine. After 1 year using RHINISENG® were examined again 35 nasal turbinates with the same method used in the 1 year ago examination. The turbinates examination was performed for the same technician in order to avoid any bias due to the interpretation of the method.
Results: Statistical differences in prevalence ($p<0.05$, Chi-square test) and nasal score ($p<0.05$, Mann-Whitney test) between periods were observed. Prevalence: Vaccine A 85,2%, Rhiniseng 62,5%. IRA: Vaccine A 1,59, Rhiniseng 0,97.
Conclusion: Based on the data presented, RHINISENG® vaccination program implemented in a farm already vaccinating regularly with an AR vaccine, and affected by a chronic Atrophic Rhinitis was able to reduce significantly the nasal lesions prevalence and the AR nasal lesion index (IRA) at slaughterhouse. RHINISENG® field efficacy has already been demonstrated in previous studies (5).

Disclosure of Interest: None Declared

Keywords: atrophic rhinitis, nasal lesion, vaccine efficacy

Vaccinology & Immunology

PO-PF3-237

Extracellular production of xylose-binding lipoprotein from Mycoplasma hyopneumoniae in Bacillus subtilis

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Introduction: *Mycoplasma hyopneumoniae* is the primary etiological agent of swine enzootic pneumonia (SEP), one of the most prevalent respiratory diseases in pigs worldwide. Vaccination is considered as an important and effective tool to control SEP. Commercially available vaccines against *M. hyopneumoniae* infection comprise inactivated whole-cell bacterins. However, production of these vaccines employing the complicated cultivation method are expensive and time consuming. The use of specific antigens as subunit vaccines produced by recombinant DNA technology may be a preferable alternative to the use of bacterins. Our previous study showed that xylose-binding protein (XylF) is a potential subunit vaccine candidate. The gene encoding XylF was cloned and expressed in *Escherichia coli*. However, the purification of recombinant XylF (rXylF) from *E. coli* is time-consuming. In the present study, we demonstrated that *Bacillus subtilis* is an attractive alternative for high-level expression and simplified purification of rXylF.
Materials and Methods: Silent mutated XylF gene which the nonsense TGA codons in the gene had been converted to TGG codons (tryptophan) was amplified by PCR from pET-XylF and cloned into three different secretion plasmids. The resulting plasmids, pSP1-XylF to pSP3-XylF, were transformed into *B. subtilis* WB800N/pBL1, respectively. Transformants were incubated with shaking at 30°C to an OD_{600} of 0.4-0.5, followed by induction with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 48 hours at 30°C. The supernatant samples of *B. subtilis* transformants were collected and analyzed by SDS-PAGE and western blot. The rXylF produced by *B. subtilis* (pSP3-XylF) was purified from culture supernatant by immobilized metal-ion affinity chromatography (IMAC). Purity of rXylF was determined by SDS-PAGE.
Results: A 1.3-kb DNA fragment encoding mature XylF with a C-terminal His-tag was cloned into three secretion plasmids. The resulting plasmids were transformed into *B. subtilis,* respectively. The expression of the rXylF by *B. subtilis* transformants were achieved by addition of IPTG. After 48 hrs of IPTG induction, a significant amount of rXylF accumulated in the supernatants of all transformants were observed. The highest secretory expression level of rXylF was achieved when the XylF fused to the SP3 signal peptide. The rXylF was purified from the supernatant of *B. subtilis* by using IMAC with a yield of 19.81 mg/L and a purity of >95%.
Conclusion: This study presents the extracellular production and purification of rXylF in *B. subtilis*. The purified rXylF will be further used in development of a subunit vaccine or cocktail vaccine.

Disclosure of Interest: None Declared

Keywords: Bacillus subtilis, Mycoplasma hyopneumoniae , xylose-binding lipoprotein

Vaccinology & Immunology

PO-PF3-239

Serological response to PCV2 and the detection of PCV2 viremia after vaccination with different PCV2 vaccines

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Introduction: Measuring the level of PCV2 virus and/or the level of IgG/IgM in blood samples can be used to assess if pigs are infected or not during the later stages of fattening. The purpose of this case study was to investigate if PCV2-virus is detected or serologically active in fattening herds with pigs vaccinated at 4 weeks (± 7 days) of age with Porcilis® PCV, CircoFLEX, Circovac or Suvaxyn PCV.
Materials and Methods: In seventeen herds vaccinating with Suvaxyn PCV, twelve herds vaccinating with Circovac, twelve herds vaccinating with Porcilis® PCV and eleven herds vaccinating with Ingelvac CircoFLEX, five blood samples were taken at approx. 16 - 17 weeks of age (12 - 13 weeks after vaccination) and five blood samples were taken at approx. 21 - 22 weeks of age (18 - 19 weeks after vaccination) in the fattening unit. The samples originated from clinically healthy pigs and were tested for IgM/IgG in Ingenasa, Ingezim Circovirus IgG/IgM, and by qPCR for the presence of viral DNA. In total 2*5 samples and 2 pools of five per herd were tested.
The primary immune response leads to a detectable IgM level that is replaced by a secondary IgG response. In the present cases, the first age group was sampled at least 70 days after vaccination and the IgM values should theoretically be negative, if the vaccination provided full protection.
For the PCR test, negative results were expected to be a more common result if vaccinated pigs were fully protected following vaccination.
Results: In the Porcilis® PCV vaccinated pigs, eleven out of twelve herds had IgG positive samples, two herds had IgM positive samples, but no herds were PCV2 positive by PCR. In the CircoFLEX vaccinated pigs, nine out of eleven herds had IgG positive samples, no herds were IgM positive, but two herds had PCV2 positive PCR results. In the Circovac vaccinated pigs, eleven out of twelve herds had IgG positive samples, six herds had IgM positive samples and three herds were PCV2 positive by PCR. In Suvaxyn PCV vaccinated herds, fourteen out of seventeen herds were Ig G positive, eleven herds had IgM positive samples and eight herds were PCV2 positive by PCR.
Conclusion: In conclusion, the Porcilis® PCV herds remained PCV2 virus negative and only a few herds had a primary serological response. In comparison, a few CircoFLEX herds were PCV2 virus positive, while Circovac and Suvaxyn PCV herds appeared the least protected as indicated by IgM response and PCV2 viremia. These results may be indicative of a difference in the protective capabilities of PCV2 vaccines.

Disclosure of Interest: P. Astrup Conflict with: Employee at MSD Animal health

Keywords: Efficay, PCV2 vaccines, Viremia

Vaccinology & Immunology

PO-PF3-243

Evaluation of Interleukin-10 and other Cytokines Following PRRSV Vaccination

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Introduction: Immune-modulation by PRRSV involves the production of cytokines; however, there is a paucity of information regarding the serum concentrations of IL-10 and other cytokines in PRRSV-vaccinated pigs under real-life, farm conditions. We used vaccination with MLV as a starting point to mimic viral infection. The primary objectives were to establish IL-10, TNF-α, and TGFβ concentrations in pigs following vaccination with MLV, and to determine if changes cytokine concentrations are indicators of PRRSV-induced immunosuppression under field conditions.
Materials and Methods: The study was conducted in 3 commercial sow farms. One farm used the Ingelvac MLVÒ vaccine when pigs were 7 d of age (n=40 pigs, 3-4 pigs/litter). The second farm used the FosteraÒ PRRS vaccine at the same age (n=30 pigs). The third farm (Control; n=10 pigs) was PRRSV negative and did not use vaccine. Blood samples were collected prior to injections, and 2, 7 and 14 d after injection. Blood samples were used to determine IL-10, TNF-α, and TGFβ concentrations by enzyme immunoassay techniques. The statistical analysis included treatment, time and interactions in the model. Means were compared with Tukey's test.
Results: There were no differences in IL-10 concentrations among the three groups of pigs. The IL-10 concentrations were 21.6±5.4 pg/ml and 17.8±4.1 pg/ml prior to vaccination and at 14 d after vaccination, respectively. In contrast, TNFα levels were lower (P<.05) in control pigs than the vaccinated pigs at all days after vaccination. The TNF concentrations increased from 38.9+14 pg/ml prior to vaccination to 53±19, and 76±27 pg/ml at d 7 and 14, respectively, after vaccination. The TNF concentrations remained below 43 pg/ml in control pigs. Both vaccines resulted in increases (P<.05) in TGFβ concentrations from 16±4.2 pg/ml prior to vaccination to 24±5 pg/ml at d 14. These TGFβ concentrations were greater (P<.05) than those in the control animals at d 2, 7, and 14.
Conclusion: The two modified live vaccines stimulated an immune response within 2 days after vaccination. By 7-14 d, this response was characterized, at least in part, by increased TNFα and TGFβ. The TNFα stimulates responses to decrease viral replication and initiate the adaptive immune response. It was apparent that IL-10 release was not involved in the response to the PRRSV vaccines. In contrast, the vaccines stimulated TGFβ release. The TGFβ is released from T_H2 cells, and is inhibitory to B-cells and other T cells, and inhibits activation of macrophages. Collectively, TGFβ may act as an immuno-suppressive factor to regulate the immune response.

Disclosure of Interest: None Declared

Keywords: Cytokines, PRRSV, Vaccination

Poster Abstracts

Vaccinology & Immunology

PO-PF3-244

FIELD STUDY COMPARING TWO NEONATAL DIARRHOEA VACCINES IN BRAZIL

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Introduction: INRODUCTION
The aim of this study was to compare the production parameters in the farrowing units of a Brazilian commercial farm suffering recurrent neonatal diarrhoea, during two 10 months consecutives periods using two different vaccines against neonatal diarrhoeas. Two different vaccines against neonatal diarrhoeas were utilized in each of the periods compared. In the first period, sows were vaccinated with a bacterin vaccine which included different *E.coli* strains (Vaccine A) and in the second period sows were vaccinated with SUISENG® which contains purified adhesion factors (F4ab, F4ac, F5 and F6) and the heat labile toxin (LT) of *Escherichia coli.*

Materials and Methods: MATERIALS AND METHOD
The study was carried out in a 1000 sow's farm in Mato Grosso state, Brazil. The vaccination protocol included a commercial inactivated *E. coli* vaccine (Vaccine A). The main concern in the farrowing units was neonatal diarrhoea appeared in piglets after 3 day of life. Crushed piglets, starvation and diarrhoea were the main causes of death. The Colibacillosis diagnosis of the diarrhoea was carried out by a multiplex PCR. This PCR is able to detect different Coli adhesins factors linked with virulence (F4, F6, F5) and toxin β and α produced by different types of *Cl. Perfringens* (3). Sampling was performed using fecal samples from piglets showing acute signs of the diarrhoea. *E. Coli* F4 and F6 adhesins were detected from the samples, so Colibacillosis by *E.coli* was confirmed.
Vaccination using SUISENG® was implemented at the beginning of October 2014 and the vaccination program used was two doses in gilts, one dose 8 weeks before farrowing and a revaccination 4 weeks later; and in multiparous sows a booster dose was administrated 4 weeks before the subsequent farrowing.
In order to assess the field efficacy of SUISENG® data of piglet mortality, piglets weaned per litter, weaning weight and ADG were reported during the two periods analysed.
Results: Statistical differences between periods were observed in all the parameters evaluated. ($p<0,05$, t-test for independent samples). Mean of piglet mortality rate: Vaccine A 5.84, Suiseng 3.84. Mean body weight at weaning: Vaccine A 5.46, Suiseng 5.89. ADG mean during lactation period: Vaccine A 0.19, Suiseng 0.21.
Conclusion: CONCLUSIONS AND DISCUSSION
Based on the data presented, SUISENG® vaccination program implemented in a farm affected by a recurrent o persistent Colibacillosis was able to prevent the negative effects of *E. coli* infection in suckling piglets; thus improving pre-weaning mortality, the weaning weight and consequently the ADG during the lactation period.

Disclosure of Interest: None Declared

Keywords: Colibacillosis, Neonatal diarrhoea

Vaccinology & Immunology

PO-PF3-254

Field safety comparison of ERYSENG® PARVO LEPTO with two commercial vaccines.

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Introduction: The aim of this study was to compare the safety of a new octavalent combined parvovirus, erysipelas and leptospira vaccine (ERYSENG® PARVO LEPTO) versus two different commercially available vaccines and a negative control group, by evaluating the body temperature increase induced after the primary vaccination in gilts under field conditions.
Materials and Methods: A multicentric, randomized, blinded and controlled field trial was performed on 3 commercial farms located in Brazil. A total of 326 nulliparous gilts, approximately 6 months old, were randomly assigned to four different groups: group 1 (n=111) was vaccinated with a 2 ml/dose of ERYSENG® PARVO LEPTO, group 2 (n=33) was vaccinated with a 2 ml/dose commercial vaccine, group 3 (n=96) was vaccinated with a 5 ml/dose commercial vaccine and group 4 (n=86) was the negative control group. Groups 1 to 3 were immunised twice intramuscularly at 6-8 weeks before mating and 21 days after the first dose. Animals in group 4 received 2ml/dose of phosphate buffered saline (PBS) following the same schedule as groups 1 to 3. Rectal temperatures were recorded individually on the day of vaccination, 6h post-vaccination and 24h later. Statistical analyses were undertaken using the Mann-Whitney test for body temperatures ($p<0.05$).
Results: After vaccinations, a transient rise in temperature occurred 6h post inoculation in all vaccinated groups. All groups were within the physiological range (±0.5 ºC) except group 2, which increased by an average of 1.02ºC at d0+6h and 1.13ºC at d21+6h. ERYSENG® PARVO LEPTO and group 3 remained below group 2. These groups showed a maximum mean increase of 0.33ºC at d21+6h and a maximum mean increase of 0.16ºC at d21+6h, respectively. During the 1st immunization, all groups returned to physiological values within 24h of vaccination. However, with the 2nd dose, while ERYSENG® PARVO LEPTO only showed statistical differences to the control group 6h after vaccination, group 2 presented significant differences 6h and 24h after inoculation.
Conclusion: The increase in body temperature after vaccination with ERYSENG® PARVO LEPTO was within the physiological range, demonstrating the safety of the vaccine.

Disclosure of Interest: C. F. C. Scherer Conflict with: Hipra, A. Puig Conflict with: Hipra, A. Camprodon Conflict with: Hipra, M. Coll Conflict with: Hipra, M. Cesio Conflict with: Hipra, R. March Conflict with: Hipra

Keywords: Leptospira sp, safety, Vaccination

Vaccinology & Immunology

PO-PF3-260

Safety evaluation of a combined Porcine circovirus type 2 and Mycoplasma hyopneumoniae vaccine in breeding and lactating sows and gilts

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Introduction: Porcine circovirus type 2 (PCV2) is known to be one of the major swine diseases worldwide and PCV2 sow vaccination might have a positive effect on sow herd productivity. Boehringer Ingelheim has recently licensed Ingelvac CircoFLEX® for pregnant and lactating sows. As Ingelvac CircoFLEX® is often used in combination with Ingelvac MycoFLEX®, which is not registered for use in sows, it was the objective of this study to determine under field conditions whether the inadvertently application of Ingelvac CircoFLEX® mixed with Ingelvac MycoFLEX® (FLEXcombo®) is safe in pregnant or lactating sows and gilts.
Materials and Methods: The study was designed as randomized, negative controlled, blinded side-by-side study using physiologic salt solution as control product (CP). The farrow-to-finish farm chosen was antibody positive for PCV2 and positive but clinically stable with regard to Porcine reproductive and respiratory syndrome virus. In total, 176 healthy animals in the 1st, 2nd or 3rd trimester of pregnancy or in lactation were included in the study and vaccinated intramuscularly with a single dose (2 ml) of either FLEXcombo® or CP.
Results: Local reactions were observed in both treatment groups. The predominant finding was a reddening of the injection site, which was observed with similar frequency in both groups after vaccination. A transient swelling with a maximum diameter of 1.0 cm was recorded for two vaccinated sows (1st trimester). The occurrence of clinical signs like recumbence, reduced appetite or skin scratches post treatment was slightly lower for vaccinated animals (8 out of 88; 9%) than for animals having received the CP (11 out of 88; 13%). The reproductive performance of the vaccinated animals and the growth of their progeny were not negatively impacted, irrespective of the time of vaccination during pregnancy or lactation.
Conclusion: In summary, the application of FLEXcombo® was well tolerated. We conclude that the inadvertent application of the associated use of Ingelvac CircoFLEX® and Ingelvac MycoFLEX® is safe for breeding and lactating sows and gilts under field conditions.

Disclosure of Interest: None Declared

Keywords: PCV2 vaccine, safety, sow vaccination

Vaccinology & Immunology

PO-PF3-261

Relative quantification of inactivated FMDV antigens in commercial FMDV vaccines

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Introduction: Foot-and-mouth disease virus (FMDV) is one of the most infectious viral pathogen which causing massive economic loss in susceptible animals. For successful control and prevention of FMDV, vaccination is regarded as an important tool. However, commercial FMDV vaccines have been employed protective dose (PD_{50}) in cattle as a standard unit for the antigen content in the killed vaccine. Nevertheless, the unit is relatively subjective because of variation in various animal species. Furthermore, the PD_{50} were varied depending on the FMDV strains. Therefore, standardizing methods which could indicate correct antigen payload of post-manufactured vaccines should be established. In this study, inactivated FMDV antigens contained in commercial vaccines were identified and quantified by nucleic acid detection methods.
Materials and Methods: Both vaccine A and vaccine B used in this study were inactivated multivalent vaccines (O, A, Asia1). Gene recovery was evaluated by calculating the PCR efficiency in various preparation for accurate analysis. In the most efficiently amplified method, identification and quantification were carried out using qPCR methods targeting the coding sequence of structural protein VP1. For absolute quantification of FMDV, qPCR product of serotype O were inserted into plasmid DNA for internal amplification control.
Results: As a result, two vaccines had difference in identity and quantity of antigen strains. The partial nucleotides of VP1 of serotype O contained in FMDV vaccines indicated 100% identity with O1manisa for serotype O of vaccine A whether 89% identity in vaccine B. Other serotypes, A and Asia1, showed identical identity between two vaccines which represented as 98% identity with A Malaysia97 and 100% with Asia1 shamir. The Cq values of inactivated FMDV antigens were determined by each strain specific 5' nuclease-based qPCR. In case of serotype O strains of each vaccines, antigens were quantitated as 9.63±0.01 (Log_{10}) for vaccine A and 8.67±0.03 (Log_{10}) for vaccine B, which indicated that vaccine A contains about 10 times more antigen payload of serotype O than vaccine B.
Conclusion: FMDV strains in the two commercial vaccines were identified and quantified using PCR methods despite the vaccines were inactivated by binary ethylenimine and processed for purification of antigen. Furthermore, differentiated analysis was possible between and within serotypes which belong to the same cluster of topotype. Therefore, the qPCR methods could be useful for the quantification of inactivated FMDV, moreover, for other diseases.

Disclosure of Interest: None Declared

Keywords: FMD, qPCR, Vaccine

Poster Abstracts

Vaccinology & Immunology

PO-PF3-262

The effect of amoxicillin, ceftiofur, doxycycline, tiamulin and tulathromycin on swine humoral immune response induced by erysipelas vaccination

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Introduction: Antibiotics are widely used in veterinary medicine, but it has been shown that beyond antimicrobial properties they can influence the host immune system. In the present study the effects of normal chemotherapy with amoxicillin (AMX), ceftiofur (CEF), doxycycline (DOXY), tiamulin (TIAM) and tulathromycin (TUL) on the postvaccinal immune response after vaccination of pigs against erysipelas with inactivated vaccine were studied. Vaccination is the most common tool used for the prevention of swine erysipelas in the field condition and humoral immunity is considered as the most important in the protection against this disease. The present study was focused on the interactions between antibiotics and humoral postvaccinal immunity.
Materials and Methods: One hundred and five, 8-week-old pigs, both sexes were used. Animals were sourced from herd with high health status. Only pigs that not received antibiotics before study were involved in the experiment.
Animals were divided into 7 groups (n=15). Pigs from 5 groups received AMX, CEF, DOXY, TIAM or TUL respectively, according to the recommended doses. Pigs from AMX, CEF, DOXY, TIAM, TUL and control vaccinated (CV) group were vaccinated intramuscularly (twice) against erysipelas with commercially available inactivated vaccine. The animals from group (C) were not vaccinated and did not receive antibiotics. The commercially available products containing antibiotics were used.
Pigs were bled every 14 days throughout the experimental period and the humoral immune responses were examined using an ELISA assay (CIVTEST SUIS SE/MR, HIPRA).
Results: In pigs treated with DOXY, CEF and TIAM the significant reduction regarding the ELISA score as well as percentage of positive pigs was observed after vaccination. In contrast, in pigs vaccinated in the face of AMX or TUL the stronger, long-lasting humoral response was found.
Conclusion: Results of the present study indicate that interactions between immune system and antibiotics should be taken into account when planning the vaccination in the pig herds. Vaccination in the presence of antibiotics may result in a modulation of specific antibodies production and in this way may alter the protection against infection.

Disclosure of Interest: None Declared

Keywords: antibiotics, humoral immunity, Vaccination

Vaccinology & Immunology

PO-PF3-268

PRRSV spreading in swine herd after mass vaccination of breeding stock

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) caused by the PRRS virus (PRRSV) is currently one of the most important diseases affecting pigs and causes significant economic loss in swine industry worldwide. One of the technique proposed to control PRRS is vaccination with modified live vaccines (MLV). The aim of this study was to assess the PRRSV spreading within the herd, after introduction of vaccination with MLV in breeding animals.
Materials and Methods: The field trial was settled in a farrow-to-finish farm with 60 sows without: batch production system and AI/AO principle and with poor biosecurity and sanitary level. Based on positive serological results (PRRSV seroprevalence – 71.4%) and clinical signs (retardation growth, coughing, pneumonia) vaccination against PRRS was implemented. Due to the fact that clinical signs were observed in pre- and postweaning pigs it has been decided to introduce mass vaccination in breeding stock. The vaccination protocol was as follows: two mass vaccination of sows, gilts and boars with MLV with four weeks interval and thereafter one MLV injection every 4 months. Incoming gilts entering the quarantine were vaccinated twice with three weeks interval.
In each sampling, blood were taken from 10 animals per age category (sows, and pigs aged: 2-3; 4-5; 7-8; 10-11; 13-14; 16-17;19-20 weeks). Blood collection was performed one day before 1st and 2nd vaccination and 2 and 4 months after 2nd vaccination.
For detection of PRRSV genetic material EZ-PRRSV™MPX 4.0 Real Time PCR Kit, (Tetracore) with quantification standards was used.
Results: First sampling outcomes indicated that viremia was present in pigs aging from 2 to 9 weeks. Four weeks after the 1st vaccination PRRSV positive animals were present only in pigs from 10 to 11 weeks. However, two and four months after 2nd mass vaccination viremia has been again observed in pigs aged 7-11 and 4-8 weeks, respectively. In all positive sera PRRSV EU type was found.
Conclusion: The results of the presented study indicated that mass vaccination implemented in breeding animals delayed the development of viremia in piglets. However, it may be suggested that under current field conditions (continuous production flow, poor biosecurity and sanitary level) a four month interval between vaccinations may be too long to efficiently control the PRRSV infection at the herd level.

Disclosure of Interest: None Declared

Keywords: field conditions, MLV, PRRSV

Vaccinology & Immunology

PO-PF3-274

Immune modulating effect of a seaweed extract on specific IgG and total IgA titers in the colostrum and milk in gilts

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Introduction: The immunomodulation potential of a crude extract, from green algae Ulva armoricana (EA), has been demonstrated in vitro by Berri and al (2015). The goal of the present study was to evaluate in vivo the immunomodulation effect of several EA concentrations, administrated orally to gilts.
Materials and Methods: In a commercial farm, 35 gilts were divided into four groups. Three groups received three different daily doses of EA: 2g (EA1), 8g (EA2) and 16g (EA3); the fourth received only the excipient (biscuit) as a control. The EA was distributed over 2 periods of three consecutive days: before the atrophic rhinitis vaccine booster and the week before the theoretical farrow. Anti-Bordetella IgG antibodies were measured in the colostrum, the milk and the blood of the gilts and total IgA in the colostrum and the milk.
Results: The kinetics between the serum taken before the farrow and the colostrum showed an increase in the IgG title in the EA3 group ($P<0.05$). A tendency was also observed in the EA2 and EA1 groups. Regarding the IgA level in the colostrum and the milk, the EA2 group showed a higher level of IgA than the control ($P<0.05$) whereas doses 1 and 3 denoted an inhibition compared with the EA2 group.
Conclusion: The results indicate that the seaweed extract administrated orally could stimulate the enteromammary immune system. Additional studies are necessary to determine the mechanisms involved.

Disclosure of Interest: None Declared

Keywords: Gilts, immunomodulation, Seaweed

Vaccinology & Immunology

PO-PF3-275

EVALUATION OF TWO VACCINATION PROGRAMS AGAINST PRRS VIRUS, PCV2 AND MYCOPLASMA HYOPNEUMONIAE IN A SWINE PRODUCTION SYSTEM IN MEXICO

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Introduction: Nowadays swine farms in México are facing challenges by multiple agents. PRDC pathogens are diagnosed in the field by Veterinarians, and control programs are established through vaccines, management interventions and strategic medications. The objective of this study is to evaluate the efficacy of 2 vaccination protocols under similar field conditions.
Materials and Methods: Pigs in a 4,200 multi-sites sow farm situated in the south of Mexico were treated under 2 different vaccination protocols. According to the standard herd classification, the farm is considered a category II-A, positive stable. 52,846 pigs, born July to November 2014 and referred to as "group A" received 2ml IM of Ingelvac® PRRS MLV and 2 ml IM of FLEXcombo® (Ingelvac CircoFLEX® and Ingelvac MycoFLEX®) at 14 and 21 days of age respectively. The other group ("group B") included 44,889 pigs which were born from November 2014 to March 2015 and received one IM dose of 2ml of 3FLEX at 21 days of age. Through BECAL (Boehringer Ingelheim Economic Calculator), the efficacy of the new vaccination protocol was calculated at the end of the trial, comparing the performance of both groups.
Results: Parameters as FCR (feed conversion ratio), ADWG (average daily weight gain), mortality and ADOF (average days on feed), were evaluated in order to compare the efficacy of the new vaccination schedule. The three mayor parameters: ADWG, Mortality and ADOF, showed a numerical difference in Group B comparing with Group A. 0.72 kg/day, 6.99 % and 142.82 days vs. 0.69 kg/day, 9.91 % and 146.32 days respectively. FCR didn't show a numerical difference between both groups (Group B 1.92 vs. Group A 1.91).
The difference observed in mortality compared with group A was 2.92%, which equals a profit of 2.42$ for each pig. The difference observed in ADWG compared with group A was 0.036 Kg/day, and equals a profit of 2.92$ for each pig. Using BECAL, the reduction in mortality of ≥2.25% and improvement in ADWG of ≥0.023 Kg/day demonstrates the efficacy and economic benefit of the product.
Conclusion: From this study we can conclude that 3FLEX® is safe and efficacious. The economic benefit of 3FLEX in comparison to the identical but separately administered vaccines can at least partly be assigned to the reduction of stress caused by multiple handling and injections.

Disclosure of Interest: None Declared

Keywords: 3FLEX, Economic benefit, performance

Poster Abstracts

Vaccinology & Immunology

PO-PF3-278

Corelation of intraperitoneal and subcutaneous challenge to use for efficacy test of swine erysipelas vaccine

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Introduction: *Erysipelothrix rhusiopathiae* (ER) is a causative agent of swine erysipelas (SE) which induces septicemia, endocarditis and death, causing great economic loss to the pig industry. To prevent the disease, live and killed SE vaccines have been used worldwide. In order to measure SE vaccine efficacy, ER (T2) challenge methods in mice have been used under regulation code of Korean Standard Assay. Whiles the same SE vaccine strain, the efficacy test has been mixed by intraperitoneal (IP) or subcutaneous (SC) challenge methods. Therefore, the aim of this study was to compare IP and SC (lyophilized, cultured) challenge methods of ER (T2) and to evaluate of SE vaccine efficacy test by different challenge methods.
Materials and Methods: ER (T2) was cultured on blood agar overnight and inoculated into Tryptic soy broth (TSB) with 0.1 % Tween 80. The broth were incubated for 18h at 37°C and mixed with 10% skim milk. The mixture was lyophilized and stored at -70°C. Eight hundred forty female ICR mice, weighing 15 to 20g were grouped into IP and SC (lyophilized, cultured) group according to commercial vaccines (Table 1). Each group (n = 60) was vaccinated with 1 dose according to manufacturer's recommendations and 20 heads were used as control. Four weeks later, vaccinated and control mice was challenged with ER (T2) strain of 100LD_{50} by IP [lyophilized (n=15), culture (n=15)] and SC [lyophilized (n=15), culture (n=15)], respectively. Titers were calculated as the 50% endpoint lethal dose (LD_{50}/ml) using the Reed and Muench method. All experiments were performed in accordance with the guidelines of animal care act.
Results: A colony forming unit (CFU/ml) of Erysipelothrix rhusiopathiae in lyophilization and cultivation were 2.0 x 10^7 and 5.3 x 10^8 CFU/ml, respectively (Table 1). The LD_{50} of IP and SC using lyophilization were 3.5 ± 0.5 and 5.2 ± 0.6. The LD50 of IP and SC using cultivated solution were 5.4 ± 0.1 and 7.2 ± 0.7, respectively (Table 1). In commercial vaccines, there is no almost difference between IP and SC (lyophilized, cultured) inoculation method (Table 2). The r^2 value between IP and SC was 99.71 (Fig. 1a). The r^2 value between Lyophilization and cultivation was 1.0 (Fig. 1b).
Conclusion: This study demonstrated that no difference was detected between IP and SC (lyophilized, cultured) challenge method for SE commercial vaccines. Accordingly, It does not matter that IP and SC (lyophilized, cultured) are used for SE vaccine efficacy test if the ER (T2) strain is preserved in a good condition.

Disclosure of Interest: None Declared

Keywords: Efficay, swine erysipela, vaccine

Vaccinology & Immunology

PO-PF3-282

Efficacy test of novel PRRSV live vaccine candidates

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Introduction: WGV0917(KCTC 12783BP), WGV1014(KCTC 12784BP), and WGV1107(KCTC 12785BP) were originally isolated from neonatal piglets that were shown typical PRRSV infected symptoms. These strains had genetically characterized the NA (WGV0917 and WGV1014) and EU (WGV1107) strains and culturally passaged on PAM cells for adaptation and MARC-145 cells for attenuation. This study was to compare their immunogenicity of attenuated virulent PRRSV NA and EU strains with Ingelvac MLV vaccine in guinea pigs.
Materials and Methods: Animal. A total of 12 CrlOri:HA VFA guinea pigs, 4 weeks of age. All groups of guinea pigs were inoculated by IM route. Group 1 (n=3), 2 (n=3) and 3 (n=3) were inoculated with $10^{4.5}$ $TCID_{50}$ of strain WGV0917, WGV1014 and WGV1107. Group 4 (n=3) was inoculated with $10^{4.9}$ $TCID_{50}$ Ingelvac MLV vaccine. Group 5 (n=3) was injected with PBS as controls. Blood was taken on 0, 7, 14, 21 and 28 days after vaccination. Serum samples were collected and stored at -70℃ before testing with VN.
PRRSV VN titer assay. VN titers were determined by SN test on MARC-145 cells. A 2-fold diluted serum sample was prepared, and an equal volume of virus solution with a titer of 200 TCID50/mL was added to each dilution and incubated for 1 h at 37°C. The CPEs on the cells were analyzed for 7 days after inoculation. The VN antibody titer was defined as the reciprocal of the highest dilution that inhibited CPE in 50% of the inoculated wells.
Results: Our results showed that WGV0917, WGV1014 and WGV1107 began to develop VN titers at DPI 7 and the titers were significantly higher as compared to those in Ingelvac MLV vaccine group.
Conclusion: This study indicated that all of 3 novel PRRSV vaccine candidates raised enough SN titer to protect PRRSV infection.

Disclosure of Interest: None Declared

Keywords: Ingelvac PRRS MLV, PRRSV Live Vaccine, SN titer

Vaccinology & Immunology

PO-PF3-290

Immune response in concurrent infection with viral and bacterial infection (PRRS versus Haemophilus parasuis) in respiratory tract of pigs

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) is primary pathogen of pigs causing serious failure in production and reproduction of pigs. We confirm the influence of the infection and the vaccination to the immune response in the experiment *in vivo*. The immunosuppressive activity of this virus can also predispose to secondary bacterial infections. The animals infected with both pathogens develop more severe clinical signs.
Materials and Methods: We use the simultaneous infection of porcine alveolar macrophages (PAMs) with PRRSV and *Haemophilus parasuis* to confirm this fact in *in vitro* model and describe the differences in comparison to individual infection.
Results: Concurrent infection with PRRSV and *H. parasuis* increased pro-inflammatory cytokine production (TNF-alfa, IL-1 beta, IL-8) detected both on gene and protein level in comparison to the infection of PRRSV or *H. parasuis* alone. Simultaneous infection of both pathogens decreased the production of reactive oxygen species (ROS) production although the PRRSV infection alone increased this production. It indicate the inhibitory mechanisms of *H. parasuis* to the respiratory burst of phagocytes
Conclusion: We confirm the synergic activity of viral (PRRSV) and bacterial (*H. parasuis*) infection in the pathogenesis and the severity of the respiratory disease of pigs. Severe multifactorial respiratory disease in natural conditions caused by both pathogens could be a consequence of pro-inflammatory mediated immunopathology.
The results were obtained with a financial support of the project LO1218 from the MEYS of the CR under the NPU I program and the project QJ1210120 of the Ministry of Agriculture.

Disclosure of Interest: None Declared

Keywords: PRRS, Haemophilus parasuis, immune response, synergic efect, immunosuppression

Vaccinology & Immunology

PO-PF3-297

Efficacy comparison of 2 different PRRS MLV type 2 vaccines in a commercial farm in Thailand

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Introduction: The PRRS vaccines have been considered as a part of the tools to control PRRSv problems. Several commercial type 2 PRRS vaccines are available in the Thai swine industry. The aim of this study is to compare the efficacy of PRRS vaccines type 2 vaccination in piglets in a commercial farm in Thailand.
Materials and Methods: The retrospective study was observed in 1,300 sows farrow – nursery farm with conversional system located in Chonburi province, the eastern part of Thailand. Farm has been stabilized herd by mass vaccination with Ingelvac PRRS MLV for 4 years. To minimize losses in nursery and finishing period, piglets PRRS vaccination was applied at 2 weeks and PCV2 and Mycoplasma as Flexcombo at 3 weeks then pigs are weaned at 24 days of age. A total 9,028 pigs were included in this study; 4,346 pigs vaccinated by PRRS vaccine Type 2 and 4,682 pigs were vaccinated with Ingelvac PRRS MLV. Both observation groups were vaccinated at the same age and raised under the same conditions. The mortality in the finishing site farm (8-24 weeks) was recorded as the primary parameter and analyzed by Chi Square Test and Fisher's Exact Test through the SAS System.
Results: With respect to the mortality, there was a significant statistic difference between each PRRS vaccine group; $p<0.001$.The mortality of PRRS MLV Type 2 vaccine group ranged from 8.9% to 31.0% (Mean 17.23%). When the farm changed vaccine to Ingelvac PRRS MLV the mortality decreased from 7.4% to 2.9% (Mean 4.9%).
Conclusion: The results of this study demonstrated that Ingelvac PRRS MLV® vaccine had a significantly better efficacy in grower-finisher pigs. This also marks the importance of considering using specific PRRS vaccines against PRRS field virus as measured by grower - finisher mortality reduction.

Disclosure of Interest: None Declared

Keywords: Compare, mortality, PRRS vaccine type 2

Poster Abstracts

Veterinary Public Health (Food Safety)

PO-PW1-061

Detection Of Mycoplasma Hyopneumoniae Infections In Pigs Of Nepal By Elisa

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Introduction: Mycoplasma hyopneumoniae infection is one of the causes of respiratory disease in pigs which is the primary agent in enzootic pneumonia. Enzootic Pneumonia is a chronic disease with low mortality but high morbidity causing economic loss in swine production through retarded growth, poor feed conversion, and predisposal to bacterial pulmonary infections. In Nepal, pig farming is increasing widely and the respiratory and reproductivity problem is also increasing causing loss to the farmers. Therefore this study aims to reveal the status of Mycoplasma infections in pigs.
Materials and Methods: A descriptive cross sectional study was carried out in pigs of Chitwan, Kathmandu and far-western region of Nepal from January, 2014 to January 2015 at Animal Health Research Division, Nepal. Blood samples of pigs from ear vein were collected and serum was separated and stored in deep freeze at -80^{0}C until tested. Samples were tested by ELISA for the detection of the antibodies against the M hyopneumonia.
Results: Out of 384 pig sera, 59 sera were found positive, 13 sera were found doubtful indicating 15.33% prevalence. Pigs rearing under confinement (modern and semi conventional) were found significant in causing disease. Prevalence rate was higher in improved breeds compared to Local breeds. Age wise prevalence showed highest rate of infection during growing period.
Conclusion: This study therefore confirms the exposure to Mycoplasma infections in pigs of Nepal. Environmental and management factors contribute a major role in introduction and spread of this disease. Further study needs to be conducted on the strains of M hyopneumonia, epidemiology and control of infection.

Disclosure of Interest: None Declared

Keywords: Mycoplasma hyopneumoniae, pig, production

Veterinary Public Health (Food Safety)

PO-PW1-283

The relationship between the status of Irish slaughter pigs detained ante mortem and their meat inspection outcome

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Introduction: Temporary Veterinary Inspectors (TVIs) detain slaughter pigs ante mortem (AM) for closer inspection if they have concerns regarding their health and welfare. In this study, we evaluated 164 detained pigs, all passed as fit for slaughter, to investigate if there is a relationship between the AM status of detained pigs and their condemnation level post mortem (PM).

Materials and Methods: Data collection took place over 5 days in a single Irish abattoir. The 1st data collection point was at AM inspection, where reasons for detaining pigs were recorded on the basis of of the TVI decision on duty. All detained pigs were tattooed with a 'special attention' (SA) number for identification on the slaughter line. The 2nd data collection point was at the TVI station where the result of the PM meat inspection e.g. condemnation status and reason for condemnation, was recorded based on the decision of the acting TVI. The % of detained pigs that were condemned PM, both fully and partially, was calculated. The primary reasons for AM detention and PM condemnation were also evaluated. The association between AM status of detained pigs and the likelihood of being condemned was analysed using univariable binomial logistic regression for each AM status. Reason for condemnation was analysed using a Chi-square test.
Results: Pigs examined in this study (n=164) represented 81% of all pigs detained during the data collection period. 54% of detained pigs were fully passed as fit for human consumption, 26% were partially and 16% fully condemned (4% of SA pigs were missed on the slaughter line). Lameness was the main reason for detaining pigs AM (37% of cases), followed by hernias (18%; 83% umbilical; 17% scrotal) and tail lesions (14%). Rectal prolapse, stressed/exhausted and posterior paralysis had a prevalence of c. 7% each. No significant relationship was found between the reasons for AM detention and the likelihood of condemnation or with the reasons for condemnation (P>0.05). However, 38% of hernia and 50% of tail lesion cases were condemned (fully or partially) due to abscesses while 40% of prolapse cases were condemned due to multiple abscesses. Abscess(es) were the most common (31%) reason for full or partial condemnation of detained pigs.
Conclusion: Lameness, hernias and tail lesions were the most common reasons for detaining pigs AM. Abscess(es) was the primary identified reason for PM condemnation. Finally, there was no relationship between reasons for detaining pigs AM and reasons for full or partial condemnation.

Disclosure of Interest: None Declared

Keywords: ante mortem, condemnation, meat inspection

Veterinary Public Health (Food Safety)

PO-PW1-284

Salmonella in caeca and ileocaecal lymph nodes from slaughter pigs in herds with high and low Salmonella sero-prevalence.

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Introduction: A Salmonella Control Programme had been operating in Ireland since 2002. The programme aims to reduce the level of salmonella in pigs sent to slaughter and minimise the risk to consumers of pig meat. Controls focus on the entire production and processing chain, comprising of pre-harvest (farm and transport) and post-harvest (lairage, slaughter and processing) components. However there is currently little evidence of any decrease in the Salmonella sero-prevalence in slaughter pigs.
Materials and Methods: Herds with the highest and lowest seroprevalences were targeted for sampling during 2012 and 2013. A total of 33 herds with the highest sero-prevalences and 11 herds with the lowest sero-prevalences were sampled by DAFM officials in six abattoirs. Caecal contents and ileocaecal lymph nodes were collected from 5 pigs in each herd of the herds and submitted to the laboratory for testing within 24 hours of collection. All samples were individually cultured as outlined in ISO 6579 / 2002.
Results: Salmonella were recovered from 25 of the 29 high sero-prevalence herds being present in caecal contents in the 25 herds and in lymph nodes in 13 herds. In the low sero-prevalence herds, Salmonella were recovered from 4 herds, being present in the caecal contents in 4 herds and in lymph nodes in 2 herds. Salmonella Typhimurium and its monophasic variants were the most frequently recovered isolates from samples.
Conclusion: The limited results here confirm that the serology regime currently employed is a success in regard to the stated aim of ranking pig herds in terms of Salmonella prevalence. The results also show that herds with high sero-prevalences carry a heavier load of Salmonella and pose a much greater risk of challenging food safety controls at abattoirs.

Disclosure of Interest: None Declared

Keywords: None

Veterinary Public Health (Food Safety)

PO-PW1-285

Antibiotic use and risk factors in Swiss pig breeding farms

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Introduction: Antimicrobials are traditionally used for treatment and prevention of diseases and in some countries also for their beneficial effect on feed conversion and growth rate. Prophylactic antibiotic use is mainly done in critical periods, in which pigs have an increased risk to fall ill. In sows, antimicrobials are used mainly against metritis-mastitis-agalactia (MMA) and diseases of the genitourinary tract, while in suckling, weaned and fattening pigs, gastrointestinal problems and lameness are the main indications. Since Switzerland is free of PRRSV and Enzootic Pneumonia (EP) and Actinobacillosis have been widely eradicated from this country, respiratory diseases are currently rare.
Materials and Methods: A survey on antibiotic use was carried out on 164 randomly selected pig breeding farms in Switzerland. Risk factors for an increased use of antibiotics were calculated using a multivariate regression model.
Results: Sows were treated with antibiotics on average 0.9 days per year, mainly due to MMA. A body condition score >3.5, bad hygiene, constipation and insufficient water supply have been identified as major risk factors for MMA and there a main cause for an antibiotic use in sows. Prophylactic antibiotic use to prevent fertility problems or MMA accounted for 23% of the antimicrobials used for sows. A high antibiotic use in sows was correlated with a significantly higher antibiotic use in suckling piglets and weaners.
Suckling piglets were treated on average during 0.5 days per suckling period, mainly due to diarrhea and polyarthritis. Purchased gilts, increased MMA rate and no “all-in-all-out” housing system were identified as risk factors. A prophylactic antibiotic use shortly after birth was carried out on 24% of the farms and accounted for 46% of the antibiotic consumption during the suckling period.
Weaned piglets were treated on average 4.4 days during the weaning period, mainly due to diarrhea, polyarthritis and wasting. Keeping stables empty <2 days after disinfection, group sizes >35 pigs, lack of biosecurity measures and no ceiling heating in the lying areas were identified as risk factors for antibiotic use in this category. A prophylactic antibiotic use at weaning was carried out in 47% of farms and accounted for 87% of the antimicrobial treatment days in the weaning period.
Conclusion: Most of the antibiotics were used prophylactically (in sows 23%, in suckling piglets 46%, in weaners 87%) and therefore there is a great potential to reduce antibiotic in the Swiss pig production.

Disclosure of Interest: None Declared

Keywords: antibiotic use , breeding farms, risk factors

Poster Abstracts

Veterinary Public Health (Food Safety)

PO-PW1-286

Antibodies against Pseudorabiesvirus, Swine Influenza A, Hepatitis E, Brucella and Salmonella in wild boar from Rhineland-Palatinate, Germany

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Introduction: The wild boar has been identified as a potential reservoir for a broad range of swine pathogens and notifiable diseases, e.g. Aujeszky´s Disease (SHVI) or zoonoses, e.g. Swine Influenza A (SIV), Hepatitis E (HEV), or both, e.g. Brucellosis and Salmonellosis. Pathogens could be carried over between wild boar and domestic pig herds or directly, by consume of game to the consumer. Rhineland-Palatinate harbours a huge proportion of the German wild boar and there is substantial exchange with Belgian, French and other German populations. The wild boar looks back on an enormously prosperous expansion in Germany since the last 70 years. High population densities in some regions provide serious concerns regarding their role in the spread of diseases.
Materials and Methods: We have analysed blood samples from 1936 wild boars, hunted in Rhineland-Palatinate between October 2011 and January 2014. Samples have been selected to allow for results, representative of the State of Rhineland-Palatinate. Antibody titres have been analysed by ID Screen® Aujeszky gB Competition, ID-Vet (SHVI), Influenza A Virus Antibody Test Kit, IDEXX®, ID Screen® Hepatitis E Indirect Mult-Species, ID-Vet, ID-Screen® Brucellosis Serum Indirect, ID-Vet and Swine *Salmonella* Antibody Test Kit, IDEXX®. Samples positive for SHVI have been verified by serum neutralisation test. Samples positive for SIV have been verified and subtypes H1N1, H1N2 and H3N2 have been further differentiated by haemagglutination inhibition tests.
Results: From the sampled wild boars 2.6% were positive for SHVI and 22.9% were positive for HEV antibodies. In both cases the probability to be seropositive rose with increasing age of the individual. 5.1% of the wild boars were antibody positive for Influenza A Virus. H1N1 was the most prevalent serotype, H1N2 was not detected. Brucella antibodies were found in 14.6% of the wild boars. Again, older individuals (27%) were more frequently involved than younger wild boars (11%). Salmonella antibodies were found in 30% of the wild boars. Highest regional frequencies reached 56%. Shotes had lower rates of infection (29%) than adults (37%).
Conclusion: Our results provide evidence for the presence of SHVI, HEV, Swine Influenza A Virus, *Brucella* and *Salmonella* in Rhineland-Palatinate wild boar populations and prove their role as a reservoir for these important pathogens.

Disclosure of Interest: None Declared

Keywords: notifyable diseases, wild boar, zoonosis

Veterinary Public Health (Food Safety)

PO-PW1-287

Distribution of Toxoplasma gondii in pigs with a high and a low dose of infection

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Introduction: *Toxoplasma gondii* continues being one of the main food safety hazards for pregnant women and immunocompromised patients. In this sense, the distribution of this protozoan within the organism and the role of the infective dose in tissue distribution are of highly interest to understand the risk associated to the parasite and to decipher the pathobiology of this infection.
Materials and Methods: To carry out this study 11 Iberian pigs (twelve-month-old, 100Kg BW, male) were randomly allocated within 3 experimental groups: (1) Control group (2 pigs); (2) High Dose (HD) (5 pigs), animals intramuscularly inoculated with $1x10^7$ tachyzoites of *T. gondii* strain BCR Reference TgH 00001 (PRU); and (3), Low Dose (LD) (4 pigs), animals intramuscularly inoculated with $1x10^3$ tachyzoites of the same *T. gondii* strain. Blood samples were collected one week prior to inoculation and at 0, 15 and 30 days post-infection (dpi). At 30dpi all the animals were euthanized and samples from the brain, heart, tongue, lung, liver, spleen, kidney, mesenteric lymph node, masseter muscle and a pool of shoulder, loin and ham (meat pool) were collected at the post-mortem examination. All organs were examined for gross lesions and subjected to qPCR (TOX4-TOX5) to determine the presence of the parasite.
Results: No changes were detected in sera from control and LD animals throughout the study, but HD animals presented between 3.8 and 16.1 fold increase in the OD (ID Screen Toxoplasmosis, IDvet). No gross lesions were detected in control or infected animals along the study. *T. gondii* DNA was not detected in control animals. *T. gondii* DNA was more frequently detected from brain and meat pool samples from HD animals (80% and 80%, respectively) than LD animals (25% and 0%, respectively). On the contrary, *T. gondii* DNA was more frequently detected from lung, liver and spleen samples from LD animals (75%, 100% and 80%, respectively) than HD animals (40%, 40% and 40%, respectively). No differences were observed in *T. gondii* distribution between HD animals and LD animals in heart, tongue, masseter muscle, kidney and mesenteric lymph node samples (100%, 40%, 80%, 100% and 100% vs 75%, 50%, 100%, 75% and 75%, respectively).
Conclusion: Our results highlight that despite the fact that *T. gondii* might present different tissue tropism according to the infective dose, target skeletal muscles are similarly affected independently of the infective dose. Therefore, different organs should be analysed to clarify the pathogenesis of this infection.

Disclosure of Interest: None Declared

Keywords: Distribution, Dose, Toxoplasma gondii

Veterinary Public Health (Food Safety)

PO-PW1-288

Title: On farm practices associated with mitigation and increased risk of Salmonella in Ireland

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Introduction: *Salmonella* is one of the most important zoonotic pathogens affecting pigs because a substantial number of human cases, including fatal cases, are linked to pork consumption. In the last two decades many countries have invested in control of the infection through surveillance and control strategies on farm. The present study aimed to find factors associated with either increased risk or reduced levels of *Salmonella* on farm using data provided by serological surveillance of finishing pigs under the Irish National *Salmonella* Control Programme.
Materials and Methods: A cross-sectional questionnaire study was conducted to collect information from Irish herds delivering finishing pigs to slaughter from October 2014 to May 2015. Meat-juice serological data from participating herds (between January and December 2014) was provided by the Department of Agriculture Food and Marine (DAFM). Annual prevalence was estimated by dividing the number of positive pigs delivered to the slaughterhouse by the total number of pigs delivered from the same herd. A univariate analysis of the variables against herd prevalence was performed and those with a *P-value* <0.25 were included in multivariable analysis to select the variables related to *Salmonella* prevalence.
Results: A total of 61 herds was included in the study. Nine of 125 variables included in the questionnaire were retained in the final model. Homemade-feed, usually in the form of meal, was associated with reduced *Salmonella* prevalence compared to use of purchased feed *(-8.427* vs. baseline). Biosecurity factors, including the presence of double fencing (no fence estimate *+5.173*), boot changing (no change of boots estimate *+18.046*) and no access to the yard by the feed truck (estimate *-10.063*) were also related to a lower *Salmonella* prevalence, while the presence of cats on farm was linked to higher prevalence. Finally, intestinal diseases (swine dysentery (estimate *+17.025*) and diarrhoea caused by *E. coli* (*estimate +10.65*)) were linked to the presence of *Salmonella,* suggesting that intestinal pathogens can act in synergy with each other.
Conclusion: These results support findings reported from other countries and show that the occurrence of *Salmonella* in pigs is influenced by many factors. Control of *Salmonella* in Irish herds should address the on-farm factors highlighted in this study, including feed, biosecurity and control of other diseases present in the herd. Control measures should also include other management and husbandry factors not detected in the present study but highlighted by previous research.

Disclosure of Interest: None Declared

Keywords: Control, Risk factors, Salmonella

Veterinary Public Health (Food Safety)

PO-PW1-289

differential interaction of gC1qR protein with the capsid proteins of porcine circoviruses.

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Introduction: Porcine circovirus type 2 (PCV-2) is the causal agent of the post weaning multisystemic wasting syndrome (PWMS). This virus is different from PCV-1 that is non-pathogenic. Globally PCV-2 strains are classified in two genogroups: a and b. PCV genome contains two major ORF that encode Rep and Rep' proteins associated to the viral replication and the capsid protein (Cap) that is the unique structural component of the virus. The Capsid protein of circovirus interacts with the cell protein gC1qR that is the membranar receptor of the globular head of the complement protein C1q. This study aimed to determine the expression level of gC1qR transcripts infected by PCV-2, to determine the region of PCV-2 Cap required for the interaction with gC1qR and to evaluate the interaction between gC1qR and different strains of PCV.
Materials and Methods: Infection of piglets and RT-PCR:
4 piglets were infected by intramuscular and intratracheal route by PCV-2.Three days after infection samples were collected from mesenteric, inguinal and tracheo-brochial lymph nodes and spleen on tonsils.The levels of gC1qR transcripts and the PCV-2 viral genomic loads were determined by real time PCR.
Yeast two hybrid assay:
DNA sequences comprising the mature proteins of gC1qR has been amplified from PK-15 cells and a spleen cDNA library and then cloned into the pGADT7 plasmid.The Cap proteins of PCV-2a,PCV-2b and two strains of PCV-1 have been cloned in the pGBKT7 plasmid.The yeast Y2HGOLD from Clonetech was co-transformed by different combination of pGADT7 and pGBKT7 constructions and plated on double drop out medium SD-2 (-Leu,-Trp) and incubated for four days at 30°C. The yeast colonies obtained were used for making patches on SD-2 medium that were further incubated for two days at 30°C and duplicated on SD-2 plates and quadruple drop out medium SD-4 for determine protein interaction.
Results: We have shown that the gC1qR transcripts are downregulated in the tracheobronchial lymph nodes of piglets infected by PCV-2, three days after infection. We have shown that the 59 N-terminal amino acids of PCV-2 Cap is required for the interaction with gC1qR. Furthermore gC1qR interacts with PCV-2a Cap, PCV-2b Cap but also with the Cap protein of a PCV-1 strain isolated in Germany. However the Cap protein of a PCV-1 isolated in France from a pig co-infected by PCV-1 and PCV-2 does not interact with gC1qR.
Conclusion: The differential interaction of gC1qR with the Cap proteins of pathogenic and non-pathogenic strains may explain their virulence. Future works will determine the importance of the interaction of gC1qR with Cap proteins during the cell infection by porcine circoviruses.

Disclosure of Interest: None Declared

Keywords: None

Poster Abstracts

Veterinary Public Health (Food Safety)

PO-PW1-290

Prevalence and Antimicrobial Resistance of Salmonella Isolated from Workers, Internal Organs and Carcasses at Slaughterhouse, Northeastern Thailand.

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Introduction: Food-borne disease caused by *Salmonella* spp. is an important public health problem. The antimicrobial resistance causes failure of regular therapy and increases the cost of treatment. *Salmonella*-free carcasses at the slaughterhouse may not happen because there are many stages where workers, liver, intestine, and pig carcasses can be contaminated.Food-borne disease caused by *Salmonella* spp. is an important public health problem. The antimicrobial resistance causes failure of regular therapy and increases the cost of treatment. *Salmonella*-free carcasses at the slaughterhouse may not happen because there are many stages where workers, liver, intestine, and pig carcasses can be contaminated. The objective of this study were to determine antimicrobial resistance of *Salmonella* spp. isolated from workers, liver, intestine and pig carcasses at slaughterhouse in Northeastern Thailand.
Materials and Methods: A total of 143 samples from workers, livers, intestines and pig carcasses consisted of were collected 38, 22, 25 and 58, respectively at slaughterhouse, Northeastern Thailand. The samplings were collected during April 2012 to September 2013. All samples were examined for *Salmonella* spp. isolation and identification by ISO 6597:2002. To assess the prevalence of antimicrobial resistance patterns was done using disk diffusion technique among 10 antimicrobials.
Results: *Salmonella* spp. contaminated to workers, livers, intestines and pig carcasses were 23.7%, 22.7%, 28.0% and 27.6% respectively. An identified serovar from workers were *S.* Rissen (45%), S. Stantey (11%), *S.* Bareilly (11%), *S.* Hindmarsh (11%), i.4,5,12:i:- (11%) and iv.43:Z4Z23:- (11%); from livers were *S.* Rissen (20%), *S.* Weltevreden (20%), *S.* Panama (20%), *S.* Kedougou (20%) and *S.* Gaminara (20%); from intestines were i.4,5,12:i:- (29%), *S.* Panama (14%), *S.* Stantey (14%), *S.* Weltevreden (14%), *S.* Kedougou (14%) and *S.* Anatum (14%); from pig carcasses were *S.* Rissen (56%), *S.* Weltevreden (19%), *S.* Stantey (13%), *S.* Panama (6%) and *S.* Virchow (6%). Ampicillin was high resistance of *Salmonella* spp. isolated from workers, livers, intestines and pig carcasses were 75%, 60%, 86% and 75%, respectively.
Conclusion: Contamination of *Salmonella* spp. isolated from workers, livers, intestines and pig carcasses at slaughterhouse may be due to improper sanitation and hygienic management. The prevention and control of *Salmonella* spp. contaminated to pork was standard slaughterhouse, good processing, hygiene, sanitation and careful handling from healthy workers. Finally, meat inspection for save to consume.

Disclosure of Interest: None Declared

Keywords: antimicrobial resistance, Salmonella spp., workers, pig carcasses, slaughterhouse

Veterinary Public Health (Food Safety)

PO-PW1-291

Effects of a natural feed additive and an in-feed antibiotic on abundance of antibiotic resistance genes in feces of weaned piglets

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Introduction: Antibiotics such as tetracyclines have been extensively used in swine production in the last decades. As a consequence, levels of antibiotic resistant bacteria in livestock have increased, which is discussed as major risk for public health. Thus, alternatives to antibiotics are urgently needed to reduce the amounts of antibiotics used in food producing animals. Natural feed additives, such as acids, phytogenics or pre/probiotics, are known to have a positive influence on gut health and performance. Here, we evaluated the effect of a natural feed additive, that combines a permeabilizing complex, a phytochemical and a blend of organic acids, on the abundance of antibiotic resistance genes in feces of weaned piglets.
Materials and Methods: After weaning, 24 Large White piglets with no history of antibiotic treatment were allocated to three groups (n=8): control group (basal diet; CG), antibiotic group (basal diet supplemented with 40 mg oxytetracycline-HCl/kg b.w./day for 10 days, thereafter basal diet; AG) or product group (basal diet supplemented with 3 kg/t natural feed additive; PG). Piglets received treatment diets for 28 days *ad libitum* and were housed pairwise. On day 0, 10 and 28, feces samples from individual piglets were collected. The animal experiment was approved by the State Veterinary and Food Administration of the Slovak Republic (No 1485/15-221).
For DNA extraction, the QIAamp DNA Stool Kit (Qiagen) was used according to the manufacturer´s instructions. Abundance of genes conferring resistance to tetracycline (*tet*A, *tet*B), sulfamethoxazole (*sul*1, *sul*2) and streptomycin (*str*A, *str*B) was determined by qPCR.
Results: Administration of the in-feed antibiotic significantly increased the levels of *tet*A in feces ($p < 0.05$). On day 10 and 28, mean *tet*A gene copies/g feces were 9.24E+06 and 7.43E+06 in the AG, respectively, while levels in the PG and CG remained between 6.34E+05 and 1.22E+06. Independent of the treatment, *tet*A was detected in all samples at all time points. This was not the case for *tet*B, which was less frequently found in feces of the PG and CG. Interestingly, levels of *sul*2, *str*A and *str*B decreased over time in the AG. A similar pattern was observed in the CG. Neither treatment nor time had a significant influence on copy numbers of *sul*1 and *tet*B.
Conclusion: In contrast to in-feed supplementation with oxytetracycline, the use of the natural feed additive did not increase abundance of the resistance gene *tet*A in feces of weaned piglets. As this product has previously been shown to enhance performance in pigs, its application might contribute to a reduced use of antimicrobials in swine production.

Disclosure of Interest: None Declared

Keywords: antibiotic resistance, feed additive, resistance gene

Veterinary Public Health (Food Safety)

PO-PW1-292

Impact Of Porcine Cysticercosis On Public Health And Food Safety In India

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Introduction: Due to consumption of Porcine Cysticercosis infected pork, a serious and life-threatening disease named Neuro- Cysticercosis has been reported in the 2-3% of weaker section of human population of rural and slum areas of Rajasthan state in India. 75% of hospitalized patients with neuro-cysticercosis were at productive age, and are frequently unable to work soon after the onset of symptoms associated with epilepsy. 90% of the pig's population is raised by weaker section in their houses and never bring their pigs to Government slaughter houses because of the fear of downgrading or confiscation and thus 80% of the pigs go to the unofficial meat distribution centers without any reliable anti-mortem diagnosis test and at such stage it may be open to abuse and risk of human consumption.
Materials and Methods: Collected serum samples from naturally infected pigs were examined by Immunoelectrotransfer blot technique (EITB) combined with the high resolution gradient gel electrophoresis and enzyme linked immunosorbent assay (ELISA).
Results: Both techniques revealed that the sensitivity of ELISA was 70% with specificity of 73% as well as 98% high sensitivity and 100% absolute specificity by EITB proved useful for ani-mortem diagnosis of porcine cysticercosis. 26% serum samples were found positive.
Conclusion: This study suggests that an awareness programme related to pork tapeworm-*Taenia solium* and zoonotic cestode infection as well as effective taenicidal drugs work against adult T.*solium* in human host and pigs with diagnostic tests must be implanted for food safety and veterinary-public health in the rural and slum areas of all over India.

Disclosure of Interest: None Declared

Keywords: Porcine cysticercosis,Diagnosis,Food safety and public health

Veterinary Public Health (Food Safety)

PO-PW1-293

Using data from a survey of swine veterinarians to identify IAV control, prevention, and research opportunities within a large multiplication system

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Introduction: In late summer 2015, the North American veterinarians of a global swine breeding stock company conducted a survey of 35 independent 'Health Team Veterinarians' (HTVs) that perform routine health monitoring for over 200 multiplication premises. The survey's content focused upon Influenza A Virus-Swine (IAV-S) which is a significant disease in the North American swine population. The participants were asked to draw upon their valuable experiential knowledge of current and historic health status, personnel, and protocol information for the premises they visit. The goal was draw upon the combined HTV knowledge to identify opportunities and direction for IAV control, prevention, and research within this North American multiplication system.
Materials and Methods: Participating HTVs were all required to complete the survey using a commercial online survey software. They were encouraged to review the survey with site managers to insure the accuracy of their responses. The expectation was that a single survey would be completed for each multiplication sow farm, boar stud, and multi-site post-weaning flow. They were reminded that, although the main findings could be shared in publications, no herd specific information would be shared so that confidentiality would be maintained.
Results: The survey was completed for 73 sites or flows. Of the 93% of HTVs that thought IAV was significant, 63% noted the lack of appropriate tools for control. 81% responded that all staff is responsible for monitoring clinical signs and 72% do it daily. Surveillance is more intensive in boar studs. A 31% of sites or flows are testing on a routine basis while the rest only test in response to clinical signs. Nasal swab is the preferred method for sampling in sow farms and boar studs while oral fluid testing is more popular in post-weaning sites. 50% of the sites have never used IAV vaccine. Of the 33% of herds currently using vaccine, a high proportion are boar studs. High variation was observed in pre-entry personnel surveillance practices, compensation programs associated with personnel sick days, and education programs.
Conclusion: The zoonotic potential of the virus and its direct economic impact for producers help to explain why such a high priority is given to understanding the virus and mitigating its impact. Survey participants provided a wealth of information that highlighted opportunities for improvements related to the control and prevention of IAV as well as opportunities for further IAV research. This survey process highlighted the value of accessing the knowledge base of a large group of practicing swine veterinarians to encourage an approach to IAV preparedness and response that is both collaborative and relevant.

Disclosure of Interest: T. Snider Conflict with: PIC North America employee, J. Geiger Conflict with: PIC North America employee, J. W. Lyons Conflict with: PIC North America employee, T. Riek Conflict with: PIC North America employee, R. Thompson Conflict with: PIC North America employee, C. Corzo Conflict with: PIC North America employee, J. P. Cano Conflict with: PIC North America employee

Keywords: IAV-S, Influenza, Survey

Poster Abstracts

Veterinary Public Health (Food Safety)

PO-PW1-294

Airborne transmission of highly pathogenic avian influenza virus. Preparedness considerations for the swine industry

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Introduction: Recently, the poultry industry has experienced severe outbreaks of the H5N2 highly pathogenic avian influenza virus (HPAIV) worldwide. Rapid spread of cases may suggest the potential for airborne spread. Based on the hypothesis that HPAIV particles can be suspended in the air and remain infectious, and the on-going threat that HPAIV could jump species and affect humans and swine, the objectives were to detect and assess the viability of HPAIV in air samples collected inside and outside affected flocks;to assess airborne particle deposition on surfaces outside affected flocks;and to characterize particle size distribution of HPAIV in air.
Materials and Methods: Six flocks located in the Midwest (US) were enrolled in the study (spring 2015). Air samples were collected inside and outside of affected flocks at 5m, 70-150m and 500-1000m, using: a) cyclonic air collector; b) size selective air samplers [8 staged Andersen cascade impactor (ACI) and 5 staged Tisch cascade impactor (TCI) collecting aerosols as a function of particle size ranigng from 0.01 to 10 microns (µm)]. Environmental samples from surfaces in locations at high risk of direct exposure to the air exhausted from the farms evaluate the risk of environmental virus deposition. All samples were tested by RT-PCR and inoculated on embryonated eggs to assess viability. Quantity of viral RNA copies per volume of air, total airborne particles, %RH and temp were analyzed at the different sampling points.
Results: A total of 138 sampling events (321 individual air samples), were analyzed by qRT-PCR. From all sampling events 67% from inside, and 45% and 4% at 5m and 70-150m, respectively, tested positive; 20% collected at 500-1000m tested suspect (low quantity of genetic material). Samples collected inside, at 5 m and at 70-150m outside the facilities had viable virus.35% of surface samples from several locations were positive but negative for virus isolation. HPAIV was also detected associated to particles of all size ranges measured inside and at 5m in quantities ranging from $1.2x10^3$ (particles between 0.4-0.7µm collected at 5m) to $1.4x10^6$ (inside particles >9µm). Overall, viable virus was detected in particles > 2.1 µm.
Conclusion: Results from this study indicate that HPAIV can be aerosolized from infected flocks and remain airborne. The transport of infectious virus airborne particles and their deposition on surfaces around infected premises appear to be a risk for the spread of HPAIV. Because of the similarities in the production systems of poultry and swine, and its zoonotic potential, this information is crucial for the preparedness and response of the swine industry and farm workers against this highly devastating pathogen.

Disclosure of Interest: None Declared

Keywords: Airborne transmission, Highly pathogenic avian influenza, Preparedness

Veterinary Public Health (Food Safety)

PO-PW1-295

Antibiotic use and risk factors in Swiss pig fattening farms

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Introduction: The use of antibiotic drugs in food producing animals is considered to be a major risk factor for selection of antimicrobial resistance in humans as well as in animals. All antibiotics used in veterinary medicine are closely related to the antibiotics used in human medicine and may induce cross-resistance. Hence, minimizing antibiotic usage and prudent use of antibiotics are two key points to minimize the development of antibiotic resistance. Antibiotics are used in fattening pigs especially to avoid diarrhea and respiratory diseases. Fear of economic losses and confirmed habit are the main reasons for a prophylactic antibiotic use. Antibiotics are applied mainly orally and in many cases they are underdosed, which promotes antimicrobial resistances.
Materials and Methods: 101 randomly selected fattening farms were visited 3 times during the fattening period to record health status, biosecurity and management measures and indications of antibiotic use. Totally 10,969 pigs were traced from the beginning of the fattening period to the slaughterhouse. Based on farm and slaughterhouse data, the fattening performance data an animal treatment index as well as risk factors for increased antimicrobial use were calculated.
Results: During the fattening period, pigs were treated on average on 4.8 days. On 35 farms, all pigs were prophylactically treated orally at the beginning of the fattening period during 7 – 14 days. These prophylactic antibiotic applications accounted for 79% of the antibiotic treatment days, followed by oral (18%) and parenteral (3%) therapeutic applications. During the fattening period of 10,969 pigs, 7.4% of the animals had to be treated because of diarrhea, followed by lameness (5.4%), cannibalism (3.2%) respiratory diseases (2.1%) and wasting (1.3%). Sulphonamid combinations, tetracycline combinations and tiamulin were the most used antibiotics. Poor biosecurity measures, inadequate water supply, bad hygiene of feeding systems and fear of diseases were figured out as major risk factors for antibiotic use.
A prophylactic use of antibiotics at the beginning of the fattening period had no positive side effect on daily weight gain and did not lead to a decreased number of orally or parenterally treated pigs during the fattening period. A prophylactic antibiotic use was correlated to a higher mortality rate during the two first weeks of the fattening period.
Conclusion: A prophylactic antibiotic use at the beginning of the fattening period had no positive side effect to daily weight gain, treatment incidence or mortality rate. Optimizing biosecurity and management measures are more effective than antibiotics.

Disclosure of Interest: None Declared

Keywords: antibiotic use, pig fattening farms, risk factors

Veterinary Public Health (Food Safety)

PO-PW1-296

Supply Chain View To Prevention Of Antimicrobial Residues In Pork

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Introduction: Consumers and important trade markets value meat without residues of antimicrobials. Preventive actions taken by the vet and the farmer are therefore essential to prevent presence of residues. This includes careful registration of use of antimicrobials, marking of treated animals, and compliance with withdrawal periods. Moreover, surveillance (own check and official samples) is needed to detect enough cases to encourage compliance.
To identify causality, findings of residues in the own check result in a follow-up visit in the herd of origin within few days. Without such a visit, the farmer is not allowed to send pigs to slaughter. However, information about the type of antimicrobial is often not available at the time of the visit, which hampers the possibilities to explain what went wrong.
A new multi-chemical diagnostic method for testing of residues - called HPLC LC-MS/MS – is on the market and is already used in the national monitoring. It has a higher sensitivity, is quicker, but also more expensive than the traditional biological method being used.
We looked into how prevention and surveillance could be further improved.
Materials and Methods: In 2015, we undertook a trial in 10 sow herds in collaboration with a veterinary practice to evaluate different tools to mark sows. The farmers' experience with respect to these tools were collected and evaluated.
We used data from the Danish surveillance for residues in pigs covering 18,000 samples annually. To identify indicators for high-risk finishing herds, we collected specific information about findings during 2.5 years - including substances and reasons for presence. This was combined with information from the Danish meat inspection database. Next, we set up a scenario tree to simulate the effect of different future risk-based scenarios.
Results: The surveillance results show a low prevalence - in particular for finishing pigs. For sows, the prevalence is slightly higher, presumably because the slaughter date is unforeseen, which points to a special need for marking of treated sows. Here, the trial showed the usefulness of the new tools, which are cheap and easy to use.
Chronic pleurisy was identified as an indicator for high-risk finishing herds (within-herd prevalence >40%). By use of that we set up a risk-based own check program.
Conclusion: This program is now being implemented by the largest abattoir company. The sample size will be halved, and more weight will be given to sampling from high-risk herds, resulting in the same number of positive samples and equal costs. The multi-chemical method will be used, which will enable more effective follow-up visits – because information about substance found will be available prior to the visit.

Disclosure of Interest: L. Alban Conflict with: DAFC gives advice to farmers and abattoirs - and I work for them, H. Rugbjerg: None Declared, J. V. Petersen Conflict with: Works for DAFC which is the industry orgainization for farmers and abattoirs, L. R. Nielsen: None Declared

Keywords: Antimicrobial residues, Prevention, Surveillance

Veterinary Public Health (Food Safety)

PO-PW1-297

Reduction of aminoglycoside resistance and ESBL producing E. coli in pigs supplemented with flavophospholipol (Flavomycin®), a longitudinal study

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Introduction: Multidrug resistant (MDR) bacteria originated from livestock production have been claimed as an important source of bacterial resistance emerging in human hospitals. As a consequence, reducing antimicrobial resistant bacteria during animal production becomes an important mission for veterinarians. Flavophospholipol (Flavomycin®) is an antibiotic supplemented in livestock feed, targeting the cell wall of gram positive bacteria and R-plasmids of *Enterobacteriaceae*. The purpose of this study was to evaluate the efficacy of Flavomycin® on reduction of MDR *E. coli* in a longitudinal study from piglet to pork.
Materials and Methods: The study was performed in a multi-site farm containing 2,000 sows. Tiamulin and amoxicillin were routinely used in the nursery till the grower period. A total of 20 preweaned piglets born in the farm were divided into two groups; Flavomycin® (10 ppm) + amoxicillin (250 ppm) + tiamulin (100 ppm) (FAT, n = 10) and amoxicillin (250 ppm) + tiamulin (100 ppm) (AT, n = 10). The antibiotic mix was administrated to all pigs from 3 till 15 weeks old. Fecal samples were collected at 5 consecutive times: from 1-3 week, 3-8 week, 8-15 week, 15-24 week and pork meat samples . A total of 300 *E. coli's* were isolated and identified using routine microbiological test. The susceptibility to 19 antimicrobials included ESBLs were automatically determined (Vitek2, BioMérieux). For genotypic characterizations, 18 resistant genes and 17 major replicons were detected by an approved PCR and multiplex PCR. The difference in resistant between both groups were analyzed by chi-square test ($p<0.05$).
Results: By phenotypic screening, the AT group had a higher number of ESBL *E. coli* in the nursery and on pork than the Flavomycin® supplemented pigs ($p<0.05$). All ESBLs were also confirmed by resistance to 1st-3rd generations of cephalosporin. Moreover, the number of tobramycin and gentamicin resistant *E. coli* in the FAT group was lower than in the AT group in the nursery ($p<0.05$). Regarding resistant genes: *aadA1, aadA2 and aadB,* were reduced in the FAT group. Also the I1-Iγ replicon was reduced in the nursery, grower and finisher. The other antimicrobial resistant genes and replicons were not significantly different between both groups. These *in vivo* results shows that Flavomycin® can help in reducing transfer of ESBL and MDR bacteria from farm to consumers.
Conclusion: There is a reduction of ESBL *E. coli*'s and *aad* gene family encoding for aminoglycoside resistance in pigs supplemented with Flavomycin® and raised under intenstive antibiotic use.

Disclosure of Interest: None Declared

Keywords: flavomycin, multidrug resistance, pigs

Poster Abstracts

Veterinary Public Health (Food Safety)

PO-PW1-298

Listeria monocytogenes prevalence, contamination pattern, and antibiotic resistance in two pork slaughter and cutting plants

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Introduction: The prevalence of *Listeria monocytogenes* (Lm) in slaughtered pigs and the contamination pattern during the early stages of ham processing chain were evaluated in two abattoirs and annexed cutting plants.
Materials and Methods: Tonsils, faeces, ileocecal limph nodes (ICL) were collected from 285 carcasses. Environment and equipment samples were collected from slaughter area (SA), cutting area (CA), chill room (CR) and trimming area (TA). Samples were also collected from hams after cutting (HAC), hams after cooling and before trimming (HBT), hams before curing (HBC). The isolates were subtyped by PFGE and Minimum Inhibitory Concentrations (MICs) were also determined by microdilution, using a commercial kit (17 antibiotics).
Results: Lm has been isolated in 3.5 % (IC 95%: 1.7%>6.3%) tonsils, 0.7% (IC 95%: 0.1%>2.5%) faeces and 3.5% (IC 95%: 0.1%>8.7%) ICLs. Overall 10 pulsotypes were detected. *Plant A:* Lm has been recovered in 1/36 (2.8%) samples in CA and 33.3% in TA. Lm has never been recovered from hams. PFGE detected 8 pulsotypes, only one detected two times. *Plant B:* Lm has been detected in 1/30 (4.4%) sample in SA (splitting saw), 42.5% samples in CA and 50% in TA. Lm has been isolated in 4/10 pools, 8/10 pools and 23% of HAC, HBT and HBC, respectively. Seven pulsotypes have been detected with 3 being predominant and persistent.
All isolates were susceptible to beta-lactams. In addition to the low susceptibility to daptomycin, ceftriaxone and clindamycin, resistance to sulfamethoxazole-trimethoprim (SXT) (3.0%), tetracycline (3.0%), erythromycin (1.5%) and fluoroquinolones (0.8%) was observed.
Conclusion: Lm has been isolated from environment in both the plants with different prevalence and this reflects in a higher risk of ham contamination, as observed in B. In this plant an increasing prevalence and genetic homogeneity of Lm strains was observed throughout the processing steps. Probably the high environment and equipment contamination in CA is the origin of cross-contamination between surfaces and fresh hams and vice-versa, with a subsequent spread and adaptation of strains downstream the processing chain. Previous studies showed the minor role of the direct carry-over of Lm from pig to end-product. Lymphatic organs could be an important source of Lm introduction in the cutting plant, as evidenced by the detection of a persistent CA pulsotype from ICLs and saw in the plant B. However this study highlights the importance of environmental Lm persistence. No resistance was observed to the common antibiotics used in early listeriosis therapy; it is noteworthy the detection of resistant strains to SXT, used as second-choice antimicrobial.

Disclosure of Interest: None Declared

Keywords: Antibiotic resistance, Listeria monocytogenes, Pork meat processing

Veterinary Public Health (Food Safety)

PO-PW1-299

Antimicrobial Resistance of Salmonella spp. Isolated from Workers and Pigs from Farms in Northeastern Thailand

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Introduction: Salmonella is a major food safety hazard applied to one health. Salmonella infection in pig farms may lead to health problems for farm workers, visitors and consumers of farm produce. In this study, prevalence of *Salmonella* spp. isolated from workers and pigs at farms at Nong-Bua-Lumphu province, Northeastern of Thailand was determined and antimicrobial susceptibility was assessed.
Materials and Methods: A total of 123 samples from workers and pigs were collected during April 2012 to September 2013. Thirty-nine were from workers and 84 were from pigs from the farms, Northeastern Thailand. All samples were examined for *Salmonella* spp. isolation and identification by ISO 6597:2002. The prevalence of antimicrobial resistance patterns was assessed using disk diffusion technique among 10 antimicrobials.
Results: Percentage of *Salmonella* contaminated to workers and pigs were 28.2 and 32.2, respectively. Identified serovars from workers were *S.* Weltevreden (18.2%), *S.* Stantey (18.2%), *S.* Panama (18.2%), *S.* Muenchen (18.2%), *S.* Derby (9.1%), *S.* Vagesak (9.1%) and *S.* Give (9.1%); from pigs were *S.* Rissen (33.3%), *S.* Panama (18.5%), *S.* Stantey (11.5%), *S.* Kedougou (11.1%), *S.* Typhimurium (11.1%), *S.* Hindmarsh (3.7%), *S.* Gaminara (3.7%), i.4,5,12:i:- (3.7%), S. Gloucester (3.7%), *S.* Bareilly (3.7%,) and *S.* Eastbourne (3.7%). *Salmonella* isolated from workers and pigs that are highly resistant to Ampicillin were 63.6% and 81.5%, respectively.
Conclusion: Contamination of *Salmonella* spp. isolated from workers and pigs may be due to improper sanitation and hygienic management of the farms, or bad personal hygiene practice of the workers. The key principles for control and prevention of *Salmonella* spp. contaminated to pigs are standard farm management, sanitation management and good personal hygiene, so that food *products* derived from *pigs* will be undoubtedly safe for human consumption.

Disclosure of Interest: None Declared

Keywords: antimicrobial resistance, Salmonella, workers, pigs

Veterinary Public Health (Food Safety)

PO-PW1-300

Combination of antibiotic treatment and skin test-based culling is a suitable strategy for on farm eradication of Brucella suis biovar 2

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Introduction: Swine brucellosis outbreaks due to *Brucella suis* biovar 2 (*B. suis* 2) occur sporadically in continental Europe. Control and eradication is based on O-polysaccharide (O/PS)-based serological tests and full stamping out of infected herds. However, these tests frequently return false positive serological reactions (FPSR) due to infections with other gram-negative bacteria sharing O/PS epitopes. Previously we proved that a skin test using O/PS free cytosolic proteins is highly sensitive and specific, as well as effective to differentiate FPSR from true positives. On the other hand, full depopulation of large herds and outdoor farms rearing endangered breeds is undesirable and antibiotic treatment could be a suitable alternative. Oxytetracycline (OTC) treatment is effective to reduce the clinical impact of the disease but studies about its efficacy in a quantitative way are lacking. Reproduction ratio (*R*) can be used to compare the efficacy of different control strategies: if Reproduction (*R*)>1, infection spreads; if *R*<1, infection fades. Using *R* estimates, we assessed the efficacy of an OTC based treatment alone or combined with skin test-based culling for eradicating *B. suis* 2 from a farm
Materials and Methods: Data from herds affected by *B. suis* 2 were used. Two strategies were evaluated: *i,* (default strategy) OTC was given in feed at 20 mg/kg BW to all animals in the herd and removal of the sows based on the normal annual replacement rate (equalling the length of the infectious period -*T*-: 749 days); *ii,* the same OTC treatment combined with the removal of skin test positive animals. The *T* for strategy *ii* was modelled based on the testing interval (ranging from 1 to 25 months) and given the estimated diagnostic sensitivity for the skin test (96.4%). A deterministic Susceptible-Infectious-Removal model was used to estimate the transmission rate parameter of *B. suis* 2 under OTC treatment (*β*). *R* for each strategy was calculated as $R=\beta*T$. Three scenarios were used: 70, 200 and 800 infected animals at the moment of the onset of the outbreak.
Results: OTC treatment alone was not effective to eradicate the infection (*R* = 1.42, 95% CI 1.35-1.49). However, if combined with skin test-based culling with a monthly interval between 1 and 10 months, *R* remained under 0.6 (range 0.06-0.59). The time required to eradicate the outbreak depended on the initial number of infected animals and the test interval.
Conclusion: Once the impact of the disease was minimized by the antibiotic treatment, testing and removing skin test positive animals every 4 months resulted in effective eradication in 1-2 years, offering a suitable alternative to full depopulation of infected herds.

Disclosure of Interest: None Declared

Keywords: Brucella suis, Eradication, Reproduction ratio

Poster Abstracts

Viral and Viral Diseases
CSF/ASF

PO-PT2-081

ASF IN SARDINIA: IS THE WILD BOAR ONLY A "GUEST STAR" ?

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Introduction: The African swine fever (ASF) virus was introduced in Sardinia in 1978 and despite the application of specific eradication plans, the infection is still present.
Several factors have been associated with ASF outbreak occurrence: socio-cultural aspects, high concentration of backyards/family farms with poor biosecurity measures, presence of illegal free ranging pigs, illegal pig holdings and consequent illegal pig movements, swill feeding, just to highlight some. The ASF virus circulation among pig farms could not be ascribed to the arthropod host, because Ornithodoros species was never detected. The wild boar population inhabits the hilly and mountainous areas that characterise the topography of Sardinia and shares their habitat with free-ranging pigs: contacts and crossbreeding between wild and domestic animals are likely, therefore ASF virus transmission from wild to domestic pig and viceversa should be considered a common occurrence. In this contest an intensive and active surveillance plan was implemented to assess the evolution of the ASF epidemiology and better understand the role of WB population in the maintenance of the infection.
Materials and Methods: In accordance to EU indications, in the last years local Veterinary Authorities intensified active and passive surveillance of suid populations with particular attention to the wild one. All serological as well as virological "positivities" were geo-referenced.
Results: Since the hunting season of 2013 the number of tested wild boars registered a significant increase. This activity gave the evidence of a dangerous level of viral circulation in the wild population following the spread observed in domestic pigs.
Particularly, the percentage of wild boars cases on the total number of ASF outbreaks was 38,06 % in 2013, 62,16 % in 2014 and 71,42 % in 2015. On the basis of geographical data analysis it was observed that territories, with ASF cases in wild boars, registered highest numbers of ASF outbreaks.
Conclusion: The results obtained strongly suggest the importance of the wild boar role in maintaining the ASF infection in Sardinia.
Looking for the first results of the illegal free ranging pig reduction plan, in addition to the swill feeding prohibition, the better implementation of the main biosecurity measures and far to express a definitive opinion, we are firmly convinced that the wild boar could play a quite active role both for the continuous increasing of its presence on the regional territory and for its high potentiality to have contacts with domestic pig population especially in some hilly or mountainous territories where breeding typology is still very traditional.

Disclosure of Interest: None Declared

Keywords: ASF Wildboar Role

Viral and Viral Diseases
CSF/ASF

PO-PT2-055

A NEW PROPOSAL TO IMPLEMENT THE ITALIAN ASF/CSF SURVEILLANCE PLAN BASED ON A NETWORK ANALYSIS APPROACH

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Introduction: African swine fever (ASF) and Classical Swine Fever (CSF) are economically devastating viral infectious diseases, producing great socio-economic impact in affected countries. The recent European epidemiological scenario makes it necessary to increase the level of vigilance to protect swine populations and related business.
For this reason, a comprehensive proposal for a surveillance plan was recently presented in Italy (with the exception of Sardinia where ASF is still endemic) to assure the ASF/CSF "free status" The main pillar of this proposal is represented by a network analysis of the national swine sector.
Materials and Methods: A description of the national pig population in terms of herd localization and animal density was performed as well as an exhaustive evaluation of pig movements, according to the national animal registry data available.
The breeding holdings were identified as having the major potential impact on "diseases spreading" whilst fattening holdings and family farms have a minor or marginal role. "Free ranging" holdings were considered at "high risk", because of the interaction between domestic and wild pigs, as well as the dealers' rest stalls, where pigs coming from different origins are commingled.
A network analysis approach was performed taking into account that the commercial flows were considered as nets where pig holdings represented "nodes" and the animal groups that have been moved represented "links". The "links" had their "weight" (number of moved animal) and an "attribute" (date); the "links" represented "contacts" (possible diseases spreading); The "nodes" have their role in the network.
Results: In 2014, the Italian animal registry recorded 94,296 pig holdings which included 2,566 free ranging units. The analytical study performed on "from and/or to" farm movements resulted in a static network constituted of 55,157 "nodes" and 63,230 "links" for a total of more than 18 millions moved pigs. Values of centrality measure were calculated for any single "node" (in-degree, out-degree, degree, closeness, betweeness). The average distance range resulted as inferior to km 50. From the epidemiological point of view, "nodes" with high centrality values (hubs or cut-node) were estimated as "high risk factors" in infectious diseases spreading.
Conclusion: This analytical approach allows a careful understanding of the "weight" of each pig holding typology, as a potential risk factor in diseases spreading. The final goal is to carry out effective ASF/CSF surveillance in the national territory, focusing on the efforts in the areas where the risk of virus spreading is higher, consequently with a considerable saving of resources.

Disclosure of Interest: None Declared

Keywords: ASF CSF Surveillance

Viral and Viral Diseases
CSF/ASF

PO-PT2-032

Defined phylogeny of subgenotype 2.1 classical swine fever viruses

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Introduction: Classical swine fever (CSF) is a highly contagious swine disease with high morbidity and mortality, featuring symptoms of hemorrhagic fever and immunosuppression. Phylogenetic analysis was extensively used to track CSF viruses and analyzing the epidemiological situation. Currently CSF viruses are classified into 11 subgenotypes in 3 genotypes, of which subgenotype 2.1 is worldwide distributed with higher genetic diversity than other subgenotypes and more sub-subgenotypes may exist in addition to previously reported 2.1a-2.1c.
Materials and Methods: To improve the understanding of the genetic diversity of subgenotype 2.1 viruses, 190-nt and/or 1119-nt full length E2 gene fragments of 39 CSFV isolates collected between 2004-2012 in two Chinese provinces were amplified and sequenced, followed by phylogenetic analysis in comparison with reference sequences retrieved from GenBank using Clustal W method of MEGA 6.06. Neighbor-joining method including Bootstrap value of 1000 repetitions was used for construction of phylogenetic tree.
Results: Phylogenetic analyses showed that subgenotype 2.1 viruses in the world could be divided into 10 sub-subgenotypes (2.1a-2.1j) up to date and 39 isolates collected in this study were grouped into 7 of them (2.1a-2.1c and 2.1g-2.1j). Among the 10 sub-subgenotypes, 2.1d-2.1j were newly identified and sub-subgenotypes 2.1d circulated only in India, however the rest 9 were from China with some of them closely related to those from European and neighboring Asian countries. According to the temporal and spatial distribution of subgenotype 2.1 isolates, the classified 10 sub-subgenotypes were further categorized into three groups: dominant sub-subgenotype, minor sub-subgenotype and silent sub-subgenotype, and each sub-subgenotype can be assigned to certain geographical areas.
Conclusion: In general, the genetic diversity analysis of CSFV subgenotype 2.1 in the present study have update our understanding about the epidemiological situations of the subgenotype 2.1 in the world. Furthermore the comprehensive analysis will also provide new insights into the prevention and control of CSF in China and other related countries.

Disclosure of Interest: None Declared

Keywords: CSFV; subgenotype 2.1; phylogenetic diversity

Viral and Viral Diseases
CSF/ASF

PO-PT2-033

In vitro adaptation and genomic sequencing of a field classical swine fever virus strain

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Introduction: Classical swine fever (CSF) is a highly contagious swine disease and still causes substantial economic losses in the pig industry in China.
Materials and Methods: An CSF outbreak of in a pig farm was reported to our laboratory and the kidney specimen of a CSF suspected piglet at 30-day old was collected and sent to our laboratory for diagnosis using E2 gene RT-PCR followed by sequencing. In addition, PCR discrimination against PCV-2, PRRSV, BVDV, PRV and PPV was performed. After diagnosis, the field CSF virus was isolated on PK-15 cells and then adapted in the cell line by continuous cell-passages and virus-passage to yield high infectivity titer. At the end of the study, the complete genome sequence of cell adapted isolate after 46 passages (F46) was obtained by above RT-PCR using seven primer pairs covering whole genome. The amplicons were sequenced and assembled into a complete genome sequence.
Results: In result, a field CSF virus named as GD53/11 was detected and then isolated from the kidney tissue. Phylogenetic analysis based on the full-length E2 gene sequence revealed that the GD53/11 belongs to sub-subgenotype 2.1c. After adaptation in PK-15 cells the titer of the isolate was significantly increased from $10^{3.39}$ $TCID_{50}$/ml at passage 6 (F6) to $10^{8.5}$ $TCID_{50}$/ml at passage 46 with the peak titer obtained at 48 h post inoculation. Sequence comparison revealed that the E^{rns} gene of passages 6, 15, and 25 of GD53/2011 was identical to that in the original tissue, but one amino acid substitution (S476R) was detected at passages 35 and 46. Furthermore, E2 gene sequences at passages 6, 15, 25, 35 and 46 was found identical to that in the original tissue, indicating that the E2 gene is stable during CSF virus adaptation in PK-15 cells.
Conclusion: In present study a field CSFV, named GD53/2011 was isolated, fully sequenced and adapted to high growth titer in PK-15 cells, which might be a suitable isolate for future studies on CSFV infection, replication, and vaccine development.

Disclosure of Interest: None Declared

Keywords: CSFV; field isolate; cellular adaptation;

Poster Abstracts

Viral and Viral Diseases
CSF/ASF

PO-PT2-102

Studies on low virulence African swine fever virus strain of Brazilian isolates to approach the surveillance program

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Introduction: African swine fever (ASF) disease was eradicated from domestic swine herds in Brazil in 1984, after six years of hard work, determination and with highly cost due to the occurrence in pig small farms. The ASF virus (ASFV) belongs to the family Asfarviridae being the only member of Asfivirus genus. Our previous studies on ASFV epizootiology and virulence demonstrated that virus has dispersed from the first ASF outbreak in pig herds in Paracambi city of state of Rio de Janeiro to other regions especially to Southeastern and Southern where the first industrial pig farms were being established. And, pointed to the possibility of Brazilian ASF outbreaks were caused by low virulence ASFV strain. The high mortality rate in the first ASF outbreak was associated to the poor sanitary conditions and other concomitant infections. In Plum Island Animal Disease Center – PIADC, US the Brazilian isolates were inoculated healthy pigs where the ASF low virulence was reproduced. In order to understand, clarify and improve the prevention against virus entry in the country, this work was conducted to survey of pig herd field epidemiological surveillance records (FORMS) of pig herds in better sanitary conditions from the same period and which ASFV was isolated at least in one pig.
Materials and Methods: From 559 epidemiological surveillance records (FORMS) investigated, 283 filled out in emergency phase were analyzed. Number of passages needed for ASFV isolation by haemadsorption (HAD), description of ASF pathogenic signs, and mortality rates were compared. In PR, of 58 samples, 25 were ASFV-positive in the first, second or third passage of the leukocyte cultures, eighteen FORMS corresponding to 72 % of the positive results could be investigated and compared with the results of experimental Brazilian ASFV isolates inoculations held in PIADC.
Results: Relevant data about ASFV-positive pig herds such as the number of infected animals (1,060), animals with disease signs (91) and number of dead animals (58). The main ASF clinical sign descriptors in the FORMS were fever, anorexia, recumbence, flushing and/or cyanotic skin, diarrhea, hemorrhages in the skin, tremors, with hind legs appearing weak. The pathology was quite similar to found in PIADC. The mortality rates in the ASF outbreaks in PR from June (10%) to July (0.16%) indicated a less virulent ASF infection.
Conclusion: The sub-clinical course of ASF caused by low virulence virus strains increases the threat; because the virus can enter a country insidiously highlighted the importance of establishing and maintaining secure measures to prevent ASFV entrance into ASF-free countries.

Disclosure of Interest: None Declared

Keywords: African swine fever , low virulence strain, outbreaks

Viral and Viral Diseases
CSF/ASF

PO-PT2-079

Twenty-Two Months Of African Swine Fever In Poland. The Past, The Presence And The Future.

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Introduction: African Swine Fever (ASF) is a contagious viral disease affecting swine, wild boar and other hosts belonging to *Suidae* family. ASF is a notifiable disease seriously affecting local and international trade of swine. African swine fever virus (ASFV) was first detected in Poland in dead wild boar at the beginning of 2014. So far 77 cases in wild boar and 3 outbreaks in swine were diagnosed. Diagnosis of ASF is crucial to control the spread of the infection in populations of wild boar and pigs. Diagnostic methods include real-time PCR, enzyme-linked immunosorbent assay (ELISA) and immunoperoxidase test (IPT). The aim of this study was to summarize the current status of ASFV among population of wild boar in Poland for the last 22 months from the first diagnosed case and to draw possible scenario for the future spread of the virus.
Materials and Methods: Until October the 28, 2015 (starting from the January, 1, 2014) in total 10261 samples of blood, internal organs from hunted or died wild boar and 13638 samples from swine were collected. The sections of tissues were processed as 10 (w/v) homogenates in PBS then submitted for DNA extraction. Real-time PCR was conducted with primers and probes complementary to the conserved p72 gene sequence. ELISA was conducted using the Ingezim PPA Compac 1.1.PPA K3 ELISA kit (Ingenasa). Immunoperoxidase test (IPT) was conducted using fixed VERO infected Ba71V ASFV cells.
Results: All 77 diagnosed ASFV cases were located in the vicinity of the Belarussian border in the Podlaskie voivodeship. ASFV was not detected outside the restricted area. The conducted real-time PCR showed the presence of specific viral DNA in wild boar from 74 cases and swine from 3 outbreaks. The conducted ELISA and IPT confirmed the real-time PCR results in 6 ASF cases. However the 68th , 69th and 77th case were only serologically positive and no signal was found by real-time PCR. Passive surveillance (testing dead wild boar) revealed the annual prevalence of approximately 14% whereas active surviellance (examinations of hunted wild boar) showed a very small detection rate with the annual prevalence 0.12%. The only statistically significant seasonal difference in prevalence was found between Summer and Autumn ($p<0.001$) but only in relation to passive surveillance.
Conclusion: The incidence of ASFV in Poland had national and international consequences for swine trade and production. Taking into account the conducted study it has been shown that the ASFV spread is not so rapid as it was previously predicted. However, the newly occurred cases positive only by serological assays suggests the possible change in disease progress and complicates epidemiology and future diagnosis of ASF.

Disclosure of Interest: None Declared

Keywords: African swine fever, occurrence, Poland

Viral and Viral Diseases
CSF/ASF

PO-PT2-080

Phylogenetic Analysis Of African Swine Fever Virus Isolates In Poland On The Basis Of Multigene 505-2R Fragment.

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Introduction: African swine fever (ASF) is a contagious viral disease of pigs, wild boar and other representatives of the *Suidae* family. ASF emerged in Poland in February 2014. So far, 76 cases in wild boar (130 animals) and 3 outbreaks in backyard pig holdings have been identified. Apart of diagnostic procedures an important factor is to characterize the currently circulating ASFV isolates. The previously obtained results have shown the variable regions of ASFV genome are located within the groups of multigene families comprising of MGF100, MGF110, MGF300, MGF360, and MGF505. The aim of this study was to summarize the genetic diversity of Polish ASFV isolates on the basis of sequencing of MGF505-2R region.
Materials and Methods: Whole blood, marrow bones, kidney, liver, spleen, lymph nodes and lung samples were collected from dead or hunted wild boar and culled swine. Homogenates (10% w/v) of these tissues were prepared in PBS. Viral DNA was extracted directly from 200 µl aliquots of blood or tissue homogenates using QIAamp DNA Mini Kit (Qiagen, Hilden Germany). The primers specific to MGF505-2R ASFV sequence were designed on the basis of complete genome sequence of BA71V strain. The PCR products were purified and sequenced by CBDNA service (Poznań, Poland). The obtained nucleotide sequences of ASFV isolates were trimmed, assembled and aligned using Geneious R7 software (Biommaters, Auckland, New Zealand).
Results: The conducted phylogenetic analysis of MGF505-2R fragment provided evidence for repeated introductions of genetically distinct ASF viruses belonging to the genotype II. The nucleotide sequence identity between Polish ASFV isolates ranged from 98.26 to 100%. The largest cluster consisted of 40 sequences (39 derived from wild boar and 1 representing 3rd outbreak in swine). The second abundant group containing 100% homologous sequences comprised of 12 viruses: 11 from wild boar and 1 sequence from pigs (Outbreak 2). The DNA fragment of the virus recovered from pigs identified as the Outbreak 1 was slightly different from those mentioned above and the only identical sequence was from the Case 4 virus. Sequences representing cases 15, 17, 41, 45, 55 and 72 formed a clearly separated cluster with very high within-group genetic diversity (98.6-99.9%).
Conclusion: The conducted phylogenetic analysis of ASFV Polish isolates on the basis of MGF505-2R region showed high genetic identity of isolated viruses. However, minor diversity within the examined genetic region may suggest at least few introductions of the virus into the territory of Poland.

Disclosure of Interest: None Declared

Keywords: African swine fever , MGF505-2R, phylogenetic analysis

Viral and Viral Diseases
CSF/ASF

PO-PT2-058

Comparative validation of several real-time PCR methods for detection of African swine fever virus

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Introduction: The spread of African swine fever (ASF) in Poland and the Baltic states since 2014 has rendered ASF a serious threat also for Central and Western Europe. Apart from raising awareness and country-specific surveillance strategies, it is necessary to provide well characterized diagnostic tools for earliest possible detection of ASFV. Nowadays, direct ASFV detection is usually performed by real-time PCR. Here, we present a comparative validation of several published and commercial ASFV real-time PCR methods, including a method for simultaneous detection of ASFV and *Classical swine fever virus* (CSFV).
Materials and Methods: Based on a panel of 176 samples with pre-defined ASFV-status, two commercial ASFV real-time PCR kits and three published real-time PCR methods, including a triplex RT-real-time PCR for simultaneous detection of ASFV, CSFV and an inhibition control, were compared. The test panel consisted of proficiency test samples obtained from the EU Reference Laboratories for ASF and CSF, samples received from the Friedrich-Loeffler-Institute (Germany) and samples from our own clinical routine. We compared both the analytical and diagnostic sensitivity and specificity of the different methods, as well as their ability to detect sample inhibition by implementation of respective control assays. Furthermore, several commercial extraction kits, including those dedicated for purification of viral RNA, were compared regarding their ability to purify viral DNA.
Results: Overall, all tested real-time PCR methods proved highly sensitive for detection of ASFV. One commercial kit resulted in an increased rate of false-positives. Furthermore, inclusion of an assay to detect PCR-inhibition was important for assays which utilize a high sample volume. In our hands, the triplex RT-real-time PCR method for simultaneous detection of ASFV, CSFV and inhibition control performed best. It was further shown that commercial viral RNA nucleic acid extraction kits are well suited for purification of viral DNA.
Conclusion: Since ASF and CSF are indistinguishable by clinical symptoms alone, rapid laboratory testing for both diseases is imperative. Therefore, a combined ASFV/CSFV test following an efficient nucleic acid extraction step that purifies both viral RNA and DNA helps to accelerate and cheapen laboratory testing.

Disclosure of Interest: None Declared

Keywords: ASF, CSF, real-time PCR

Poster Abstracts

Viral and Viral Diseases
CSF/ASF

PO-PT2-056

Diagnosis of Classical Swine Fever: rapid, simple, innovative

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Introduction: Classical swine fever (CSF) is a highly contagious disease that needs to be confirmed by laboratory diagnosis due to highly variable, often unspecific clinical symptoms. Extensive serological investigations in the context of surveillance programs are required to demonstrate freedom from infection.
In the European Union, a strict '*stamping-out*' strategy is applied in case of a CSF outbreak. Given ethical and socio-economic concerns, application of marker vaccines for emergency vaccination is a promising alternative in future outbreak scenarios. The successful use of a marker vaccine requires a reliable diagnostic test to differentiate infected from marker-vaccinated animals (DIVA strategy). The most promising DIVA strategy involving the recently approved marker vaccine Suvaxyn® CSF Marker is based on the absence of a Classical Swine Fever Virus (CSFV) E^{rns}-specific immune response. QIAGEN's new double-antigen ELISA *pigtype*® CSFV E^{rns} Ab detects antibodies to the CSFV protein E^{rns} and thus addresses an alternative target protein to conventional E2-specific antibody ELISAs. It also offers the possibility to be used as accompanying DIVA test for suitable marker vaccines. The aim of the present study was to validate the test for both applications.
Materials and Methods: Sensitivity and specificity of *pigtype* CSFV E^{rns} Ab were evaluated by testing a serum sample panel comprising CSFV antibody positive and negative sera in comparison to a commercially available E2-specific antibody ELISA. Furthermore, application as a discriminatory test was validated using sera taken from animals vaccinated with the marker vaccine Suvaxyn CSF Marker as well as sera from vaccinated and subsequently CSFV challenged pigs.
Results: The new *pigtype* CSFV E^{rns} Ab proved highly specific and sensitive in comprehensive validation with CSFV antibody positive and negative sera. Compared to a commercial E2-specific antibody ELISA, *pigtype* CSFV E^{rns} Ab was more sensitive to sera obtained very soon after infection (≤21 days). The novel test reliably detected E^{rns} antibodies to a variety of isolates belonging to various CSFV genotypes. *pigtype* CSFV E^{rns} Ab showed comparable specificity for sera obtained after vaccination with the marker vaccine when compared to the only other commercially available E^{rns}-specific antibody ELISA, but demonstrated to be more sensitive for CSFV challenge sera obtained from marker-vaccinated and CSFV challenged pigs.
Conclusion: The *pigtype* CSFV E^{rns} Ab ELISA can be applied as antibody screening test in the context of CSF surveillance, but can also be employed as an accompanying differentiation test with Suvaxyn CSF Marker or another suitable marker vaccine.

Disclosure of Interest: D. Meyer Conflict with: Zentrales Innovationsprogramm Mittelstand, grant number: KF2097205MD1, S. Fritsche Conflict with: employed by the company producing the presented kit, C. Engemann Conflict with: employed by the company producing the presented kit, C. Schroeder Conflict with: employed by the company producing the presented kit, P. Becher Conflict with: Zentrales Innovationsprogramm Mittelstand, grant number: KF2097205MD1, A. Postel Conflict with: Zentrales Innovationsprogramm Mittelstand, grant number: KF2097205MD1

Keywords: Classical Swine Fever Virus Erns protein, Differentiation of infected from vaccinated animals (DIVA), double antigen ELISA

Viral and Viral Diseases
OTHERS

PO-PF3-069

Detection of porcine rotavirus group C in fecal samples using an antigen ELISA

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Introduction: Rotaviruses are one of the major agents of gastroenteritis in young children and animals, causing high mortality in the third world countries and sustained high economic losses in animal production. Up to now eight serotypes are defined (A-H). During the last years rotavirus research was mainly focused on group A rotaviruses (GARV); it has, however, become apparent that group C rotaviruses (GCRV) are also of importance within swine populations. Because the isolation of wild-type GCRV in cell culture is difficult by the lack of visible cytopathic effects, we have produced polyclonal antibodies for the detection of GCRV protein expression in infected cells.
Materials and Methods: To this end the gene segment 6 of a GCRV field strain was cloned into the expression vector pMAL-c2X and a fusion protein linked to maltose-binding protein (MBP) was produced using E.coli cells. The protein solution was injected into rabbits to produce a polyclonal GCRV specific serum. These antibodies were used to establish and validate a GCRV specific antigen enzyme-linked immunosorbent assay (ELISA).
Results: A GCRV specific antigen ELISA was established. The specificity of the ELISA was validated by polymerase chain reaction (PCR) and reverse transcriptase (RT-) PCR investigations of porcine positive field samples for coronavirus (PED), porcine parvovirus, GARV as well as dually GARV and GCRV positive samples.
Conclusion: Currently fecal field samples from different geographic areas are investigated using the in the GCRV-specific antigen ELISA to determine the prevalence of GCRV in pigs. The ELISA provides a diagnostic tool for inexpensive testing of large numbers of field samples and can supplement the existing RT-PCR methods.

Disclosure of Interest: None Declared

Keywords: ELISA, Rotavirus C

Viral and Viral Diseases
OTHERS

PO-PF3-065

A novel TK, gE and gI deleted pseudorabies virus provides complete protection against lethal challenge with the PRV variant

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Introduction: Pseudorabies virus (PRV) used to caused enormous economic losses worldwide. Due to the extensive application of effective vaccine, PRV has been well controlled. However, since late 2011, novel PRV variant has emerged in China, but traditional vaccines showed compromised protection efficacy. Thus, it is urgent to develop new vaccines based on novel PRV variants. We generated an attenuated PRV strain rSMXΔgI/gEΔTK derived from PRV variant SMX. The objective of this study was to evaluate its efficacy against fatal PRV variant challenge in pig.
Materials and Methods: Fifteen 3-week-old piglets, free of PRV, were randomly assigned to 3 groups, each containing 5 pigs. Group 1 was vaccinated i.m. with $10^{6.0}$ $TCID_{50}$ live rSMXΔgI/gEΔTK virus; Group 2 was administered i.m. with commercial Bartha-K61 vaccine ($10^{6.3}$ $TCID_{50}$/dose); Group 3 was unvaccination control. Blood samples were collected at 28 days post vaccination (dpv) for ELLISA test and serum neutralization assay (SNA). At 28 dpv, all pigs were challenged i.n. with $10^{7.0}$ $TCID_{50}$ SMX. After challenge, pigs were observed daily. The animals were weighed twice a week. At 21 days post challenge (dpc), all survived pigs were euthanized and necropsied.
Results: Post vaccination, all immunized animals showed anti-gB antibody positive, anti-gE antibody negative. The SNA titers of rSMXΔgI/gEΔTK vaccinated group were $2^{3.68}$, which was significantly higher than Bartha-K61 group ($2^{2.2}$). After challenge, unvaccinated group developed the severest symptoms, including high fever, lethargy, dyspnea, and severe neural symptoms, eventually lead to 100% mortality. Bartha-K61 group also displayed severe respiratory symptom. Further, ataxia were and convulsion were recorded. Moreover, one pig showed growth retardation and snuffling breathing. In contrast, rSMXΔgI/gEΔTK group only manifested transient fever, and recovered completely in 4-5 dpc. After challenge, Bartha-K61 group sustained 7 days growth arrest. Meanwhile, rSMXΔgI/gEΔTK group displayed no growth arrest at all. The average weight gain was 7.9kg, significantly higher than Bartha-K61 group, which gained 3.9kg in the end. The maximum shedding amount of Bartha-K61 group was $10^{5.45}$ $TCID_{50}$, which was 100 times higher than rSMXΔgI/gEΔTK group. Also, Bartha-K61 group experienced 4 days longer shedding period than rSMXΔgI/gEΔTK group.
Conclusion: rSMXΔgI/gEΔTK elicited higher neutralization antibody against PRV variant SMX than Bartha-K61 vaccine. Combining clinical symptoms, body weight gains and virus shedding, these results suggest that rSMXΔgI/gEΔTK, but not Bartha-K61 vaccine, induced full protection in growing pigs against lethal challenge of PRV variant.

Disclosure of Interest: None Declared

Keywords: PRV variant; live vaccine; protection efficacy

Poster Abstracts

Viral and Viral Diseases
OTHERS

PO-PF3-110

IDENTIFICATION OF A NOVEL SAPELOVIRUS FROM A PIG AND A COMPLETE GENOME SEQUENCE OF THE ISOLATED STRAIN 865/2015

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Introduction: *Sapelovirus* is a single-stranded, positive-sense RNA virus, belonging to the family *Picornaviridae*. Porcine sapelovirus (PSV) is associated with diarrhea, pneumonia, severe neurological disorders and reproductive failure in pigs. For the European countries, only limited data on PSV infections and circulating strains are available.
Materials and Methods: In January 2015, fecal samples were collected from diarrheic pigs at one farm infected with the porcine epidemic diarrhea virus (PEDV). Isolation of the cytopathic viral agent from the fecal samples was performed on the porcine kidney cells (PK-15), using a standard protocol. For the complete genome sequencing, RNA was extracted from the obtained isolate using the QIAamp Viral RNA kit (Qiagen, Hilden, Germany). RNA library was prepared using the Ion Total RNA Sequencing Kit v2 (Life Technologies, Carlsbad, CA). Emulsion PCR and enrichment were carried out using the Ion PGM Template OT2 200 Kit (Life Technologies). The amplified library was sequenced on the Ion PGM platform using the Ion PGM HQ Sequencing Kit and Ion 314 Chip v2 (Life Technologies). The obtained reads were mapped against the complete genome of PSV strain V13 (AF406813) and analyzed using the DNAStar Lasergene v10.1.1 (DNASTAR, Madison, WI).
Results: A cytopathic effect on PK-15 cells was observed in 72 h after inoculation. Genome of the identified PSV 865/2015 strain consisted of 7542 nucleotides, excluding the 3' poly(A) tail, and showed a typical picornavirus genome organization. PSV 865/2015 genome sequence shared only 85.5% at the nucleotide level identity with the sequence KS055217 (KJ821021) from South Korea and 85.1% with strain V13 (AF406613) detected in 2002 in Germany. PSV_P1-3-3_Coting(b1) isolate (KF463384), identified in 2007 in Spain, was the closest partial sequence similarity hit in GenBank with 93.7% nucleotide identity, but only 352 nucleotides are available for this strain.
Conclusion: In the present study, PSV 865/2015 strain was isolated in PK-15 cells and the complete genome sequence of this novel sapelovirus was determined by the Ion Torrent next-generation sequencing technology without the use of virus-specific primer sets. The genome data for the identified strain 865/2015 will enable future investigations of PSV epidemiology and evolution.

Disclosure of Interest: None Declared

Keywords: complete genome sequence, next-generation sequencing, porcine sapelovirus

Viral and Viral Diseases
OTHERS

PO-PF3-156

Significantly increased numbers of foetuses positive for porcine parvovirus (PPV) in Denmark in 2015 coincided with a shift in genotype

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Introduction: Porcine parvovirus (PPV) is a member of the family Parvoviridae. PPV is widespread in swine herds and causes reproductive failure, characterized by embryonic and fetal death, mummification, stillbirths and delayed return to estrus. PPV vaccines are successfully used worldwide to prevent reproductive failures.
DTU Vet receives annually between 50-100 submissions from aborted fetuses from Danish herds suspected of being infected with PPV. Until 2015, 2 to 5 % of these fetuses tested positive for PPV by PCR. In 2015, however, almost 13 % of the fetuses tested positive despite the number of submitted samples remained the same. One of the hypotheses was that a more pathogenic or antigenic different strain of PPV had been introduced into Danish pigs. The aim of the study was to determine the genetic variability of field isolates isolated in 2015 and to compare the sequences to archived older isolates.
Materials and Methods: A total of 15 Danish field isolates of PPV were included in the analysis. These isolates originated from diagnostic submissions between 2006 and 2015. DNA was extracted from tissue (pools of fetal liver, spleen and heart) using QiaAmp DNA Mini Kit according to the protocol supplied by the manufacturer. PCR products were obtained using a total numbers of 12 assays covering the full PPV genome. The PCR products were sent to LGC Genomics Germany for Sanger sequencing using the PCR primers. Phylogentic trees were constructed using neighbor-joining algorithm. PPV sequences available from Genbank were included as reference sequences.
Results: Two phylogenetic trees were constructed: one based on the nucleotide sequences of the coding region of VP1 and VP2 and one based on the amino acid sequences of VP1. The overall identity between the included sequences of VP1 and VP2 were 98 % on the nucleotide level and the level of identity of the amino acid sequence of VP1 was 97%.
For both trees, the sequences grouped into two defined clusters. Most of the viruses collected in Denmark 2006-2009 and two isolates collected in 2015, clustered with older German strains. This cluster has previously been defined as genotype 1 and also contain some vaccine strains. Most of the recent Danish field strains collected between 2009 and 2015 clustered together with recent German strains, including the genotype 2 reference strain 27a.
Conclusion: A significant increase in number of fetuses positive for PPV coincided with a shift from genotype 1 to genotype 2. Further studies are needed to clarify if this is a coincidence, if available vaccines have a reduced efficacy against genotype 2 strains and/or to clarify if this genotype is more virulent than typical genotype 1 strains.

Disclosure of Interest: None Declared

Keywords: Porcine parvovirus, PPV, reproduction

Viral and Viral Diseases
OTHERS

PO-PF3-176

IS KOBUVIRUS THE CAUSATIVE AGENT OF A PIG ENTERIC DISEASE?

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Introduction: Pocine kobuvirus with single-stranded RNA genome, also known as Aichivirus C, belongs to the family *Picornaviridae*. This virus was identified for the first time in Hungary in 2008. The virus was also identified in pigs of several countries with prevalence variation from 3.9 up to 100%. Virus was detected in healthy and diarrheic pigs, often with higher prevalence in diarrheic piglets. In our work we describe the detection of porcine kobuvirus in pigs of different health status and age categories. In addition, the virus 3D gene was also analysed.
Materials and Methods: Total RNA was isolated using TRIzol from 262 rectal swabs and stool samples collected from diarrheic and healthy pigs of different age on 7 farms in Slovakia in the period 2013 to 2015. Viral RNA was detected using single RT-PCR with primers flanking 495 bp fragment of 3D gene. Twenty PCR products were sequenced. The phylogenetic tree was constructed by the neighbor-joining method of MEGA6.
Results: Of 262 samples originating from the pigs of different age categories, the virus was detected in 63.1% of healthy and 62.7% of diarrheic animals. Kobuvirus RNA was slightly more often detected in piglets (70.8%) than in weaning (60.2%) and finishing (62.3%) pigs. No big differences and strong consistency in age groups were recorded in healthy versus diarrheic suckling piglets (68.6% vs 76.9%) weaning (68.8 vs 53.3%) or finishing (57.1 vs 75.9%) pigs. The analyses of 404 bp long fragment of the 3D gene revealed high genetic similarity of viral isolates (89 – 100%). Virus from healthy and diarrheic pigs was identical when it originated from the same farm. On the other hand, several viral sequences originating from the same farm were variable independently if they originated from healthy or diseased animals but this variability was in the range of phylogenetic cluster.The kobuvirus isolates originating from Slovakia were located in different phylogenetic clusters than viral isolates from Hungary or the Czech Republic. The isolates from Slovakia were rather clustered with Asian isolates than together with isolates originating from Europe.
Conclusion: The analysis of results in this study and results published by other authors did not clearly confirm that kobuvirus is a causative agent of some pig enteric disease since virus was detected in many healthy and diarrheic animals. The suckling piglets are probably more sensitive for kobuvirus infection than older pigs but more extensive study has to be carried out to draw clear conclusion. The phylogenetic analysis indicated that isolates from Slovakia were not closely related to the European isolates.
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Disclosure of Interest: None Declared

Keywords: diarhoea, Kobuvirus, pig

Viral and Viral Diseases
OTHERS

PO-PF3-024

Epidemiological characterization of swine rotaviruses in diagnostic samples from North America

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Introduction: Rotaviruses (RV) cause severe economic losses for swine producers. Three out of eight RV species (RVA, RVB, and RVC) cause diarrhea in pigs. However, the epidemiology of RV infection in swine populations is not clearly understood. In this study, the conditional odds of RVA, RVB or RVC detection were estimated among diagnostic samples from North America.
Materials and Methods: Between November 2009 and October 2011, 7,508 samples from pigs with diarrhea were submitted to the Veterinary Diagnostic Laboratory (VDL) of the University of Minnesota and were tested by RT-PCR for RVA, RVB, and RVC. A three-level mixed-effect logistic regression model (3L-MLMs) was used to estimate the association among RV species, age and geographical location.
Results: The majority of samples (82%) tested positive for RVA, RVB and/or RVC. Pigs 1-3 days old had lower conditional odds of RVA and RVB detection compared to any other age ($p<0.05$). However, pigs 1-3 days old had higher conditional odds of RVC detection compared to pigs 4-20 days old and pigs > 55 day old. Additionally, pigs between 21 and 55 days old had higher odds of RV species co-detection compared to pigs 1-3 days old. Our results also demonstrated that RV status among samples were more similar within state than between states.
Conclusion: RV species infections among swine in the US are not evenly distributed by age, and their distribution might differ between states. Our results illustrate the complexity of the RV epidemiology and highlighted the importance of integrating molecular diagnostics and novel statistical methods to better understand the epidemiology of RV infections in swine populations.

Disclosure of Interest: None Declared

Keywords: Rotavirus , three-level mixed-effect logistic regression model

Poster Abstracts

Viral and Viral Diseases
OTHERS

PO-PF3-068

Producing PRCV Serological and Virus Negative Piglets from a PRCV Infected Farrow to Finish Herd

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Introduction: Porcine Respiratory Coronavirus (PRCV) negative health status herds can be advantageous in the North America breeding stock industry. PRCV is a coronavirus that is capable of aerosol transmission through long distances. The purpose of the project was to produce PRCV virus and serologically negative piglets from a PRCV positive farrow to finish herd by removing piglets at 2-4 days of age from the farrowing rooms.
Materials and Methods: Historical PRCV diagnostics indicated that there was PRCV viral activity as early as 10-14 days of age.

Prior to the project, PRCV diagnostics were completed to determine current PRCV activity within the farrow to finish herd.

- 44 nasal swabs were taken from suckling piglets that were 2-5, 3-8 and 8-17 days of age. Samples tested negative for PRCV PCR pooled by 2.
- Twenty serum samples were collected from pigs at both 3 and 9 weeks of age. At 3 weeks of age, 19/20 pigs were serologically negative. At 9 weeks of age, 19/20 pigs were serologically negative.

During the project 66 sows were identified as potential mothers for the project. The sows were serum sampled 3-4 weeks prefarrow and all were found serologically negative. Over a 17-day time period, 85 boars were collected at 2-4 days after birth from 50 sows. Piglets were placed into Camfil L6 filtered chambers to be transferred from the farrow to finish herd to a Camfil L6 filtered off-site quarantine barn. Piglets were kept under strict isolation for 45 days following the entry of the last piglet.
Results: Fifteen days after the last piglet entered the off-site quarantine barn all piglets were nasal swabbed and serum sampled. All nasal swabs and serum were negative for PRCV by PCR and ELISA respectively. Piglets remained serologically negative through to project termination at 31 days post entry to the quarantine.
Conclusion: PRCV virus and antibody negative piglets can be produced from PRCV positive herd by removing the piglets at 2-4 days of age.

Disclosure of Interest: None Declared

Keywords: PRCV Elimination

Viral and Viral Diseases
OTHERS

PO-PF3-083

Detection of Pseudorabies Virus from Oral Fluids of Experimental Infected Pigs

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Introduction: Pseudorabies is one of the important infectious diseases, causing enormous economic losses to swine industry. Pseudorabies virus (PRV) is the causative agent that is monitored mainly from nasal swabs and tissue samples collected from pigs. Oral swabs have been widely used for the detection of classical swine fever virus (CSFV), foot-and-mouth disease virus (FMDV), porcine circovirus type 2(PCV2) and porcine reproductive and respiratory virus (PRRSV) in swine herds due to its simplicity in sampling. However, there is no report about the application of oral fluids sampling for PRV surveillance till now. In this study, we attempted to collect the oral fluids of experimental infected pigs based on the oral cotton swabs for further PRV surveillance.
Materials and Methods: The new PRV-HNX isolate was propagated in Vero cells, and the virus titer was determined. 35-day-old PRV negative pigs were randomly divided into 2 groups of 6 and treated with PRV infection (2 mL, 1×10^7 $TCID_{50}$/mL) and equal volume of DMEM, respectively. Sterilized cotton ropes were hung in the pens for about 30 min for pig biting to collect the oral fluids at 0d, 1-14d, 21d, and 28d post infection, respectively. Meanwhile, the nose swab was also collected. The oral fluids collected were freezing and thawing for three times, and then centrifuged at 8000 rpm/min for 5 min. The supernatant was used for DNA extraction. Then, the conventional gD-PCR and real-time PCR were performed to monitor the presence of PRV qualitatively and quantitatively.
Results: Conventional PCR detection indicated that PRV was positive at 1-28d post infection, while it was negative prior to infection. And the PRV was negative in control group all the time. Data from real-time PCR showed that PRV was positive at 1-28d post infection. Additionally, the copies of PRV detected were consistently increasing at 1-4d post infection, and peaked at 4d post infection. Meanwhile, the method based on oral fluids sampling can detect more viruses than from nose swabs. Clinical validation test was also successful.
Conclusion: In the present study, we first demonstrated that oral fluids collected by oral cotton swabs can be used for PRV detection. Compared with nose swab, we concluded that the oral cotton swab has higher sensitivity and superiority to be applied for clinical PRV surveillance.
Acknowledgment
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Disclosure of Interest: None Declared

Keywords: oral fluid, pseudorabies virus (PRV)

Viral and Viral Diseases
OTHERS

PO-PF3-161

Development of murine monoclonal antibodies for porcine rotavirus group B and C and use in immunoassays

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Introduction: Porcine rotavirus (PRV) is a non-enveloped icosahedral virus belonging to the genus *Rotavirus* and family *Rotaviridae*. Rotavirus genome has 11 segments which encode 6 nonstructure proteins (NSP1,2,3,4,5,6) and 6 structure proteins (VP1,2,3,4,6,7). Virion has three layers. The outer most layer comprise of VP4 and VP7 which are necessary for virus entry. The middle layer is VP6 protein, the most abundant and conserve structure protein. Base on the antigenicity and sequence of VP6, rotaviruses are divided into 8 serogroups (A-H). Serogroup A, B, C, E and H have been implicated in swine enteric disease. To date, isolates and genomic information of PRV group B and C are limited due to substantial difficulties in virus isolation. As a consequence, not many laboratory tools are available for virus detection and research. The goal of this study is producing monoclonal antibodies (mABs) against PRV B and C that can be used in diagnostics as well as research.
Materials and Methods: Molecular cloning and recombinant protein technology were used to produce mABs specific for PRV B and C. Full-length of PRV B was achieved by using full-length amplification of cDNA method. Whole VP6 of PRV B and C were amplified by RT-PCR and then cloned and expressed in a baculovirus system using Bac-to-Bac cloning and expression kit. The VP6 proteins of PRV were purified in native conditions using a Ni-NTA Purification System. The proteins then were used to produce mABs. Mouse immunization and care, fusion, and hybridoma production and maintenance were done at the Iowa State University *Hybridoma Facility. VP6-based* ELISA and IFA using PRV C-infected cells or Sf9 cells expressing VP6 of PRV B were used to screen hybridomas. Antibody-producing hybridomas were selected and cloned for further evaluation. The mABs then were verified and characterized by Western blot, isotyping, IFA, Immunohistochemistry (IHC).
Results: A 1269 nucleotide- long PRV B VP6 was achieved and used to design primers to amplify full-length VP6 of PRV B. 45-kDa VP6 proteins of PRV B and C were successfully expressed and purified.A total of 8 mABs each for PRV B and C were initially obtained based on reactivity with VP6 on ELISA. Among those, mAB 10B1, 10F7 and 10B5, 11F3 were selected for PRV B and C, respectively, as they were positive by ELISA, IFA and Western blot. Furthermore, these MAbs could be used in IHC to specifically detect PRV B or C in intestinal tissues from pigs with enteritis.
Conclusion: Murine monoclonal antibodies specific for VP6 of PRV B and C were successfully developed. The availability of these antibodies would enhance the pathogenesis study of various serogroups of rotaviruses in addition to its utility in diagnostic investigations.

Disclosure of Interest: None Declared

Keywords: porcine, rotavirus, monoclonal antibody

Viral and Viral Diseases
OTHERS

PO-PF3-114

Isolation and Analysis of Porcine Enteric Viruses in Ireland: Near Complete Genome Sequences of Porcine Astrovirus and Porcine Teschovirus

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Introduction: This study, to investigate porcine rotavirus in Irish pigs, focused on obtaining whole genome sequence (WGS) information of rotavirus (RV) and to compare these to global isolates. RVs are the most common cause of gastroenteritis among humans and animals. Rotaviruses are classified into eight groups, A – H, with porcine gastroenteritis being most commonly caused by Group A rotaviruses. This project was the first of its kind to undertake WGS of Irish RVs. During the investigation other viruses were isolated and this report concentrates on the analysis of two of these, namely, Teschovirus and Astrovirus. This is the first report of porcine Astrovirus and Teschovirus in Ireland.
Materials and Methods: Faecal samples were collected from Irish pigs (<2 months). Extracted RNA was tested for rotavirus by RT-PCR, targeting the VP6 gene. To determine the G and P types, the outer capsid proteins, VP4 and VP7, were subjected to Sanger sequencing. Eight samples were selected for WGS, based on the G and P combinations. Faecal suspensions were filtered, treated with a cocktail of Benzonase and Micrococcal Nuclease. Nucleic acid extraction was carried out using the QIAamp Viral RNA Mini kit. First and second strand synthesis and random PCR amplification for 25 cycles were performed using the Whole Transcriptome Amplification (WTA) kit. WTA products were prepared for Illumina sequencing using the KAPA Library Preparation kit.
Results: Representative samples were selected for Illumina analyses, resulting in the detection of a wide range of other viruses, e.g. Astrovirus, Enterovirus, Kobuvirus, Porcine associated stool circular virus, Rotavirus B, Sapovirus and Teschovirus. The Astrovirus and Teschovirus isolates have now been analysed in detail. From the resulting reads, three full and one partial astrovirus genome (AstV_11, AstV_12, AstV_20 and AstV_Par_11) could be retrieved. All three full astrovirus genomes had typical structures: UTR at both the 5'- and 3'-ends, a polyA tail at the 3'-ends and three ORFs. The genome of the porcine teschovirus (PTV) is 7,111bp, and the predicted locations for the proteins were annotated manually through comparison with a closely related genome sequence; Porcine Teschovirus 1 (NC_003985), which shares 82.7% sequence similarity.
Conclusion: This is the first report of WGS of enteric viruses in porcines in Ireland. The initial project provided a new understanding of how RV's evolve during infection through the porcine population and has added to the existing body of knowledge of porcine rotavirus. The unexpected discovery of a number of other viruses from the porcine samples warrants further investigation and this is currently underway.

Disclosure of Interest: None Declared

Keywords: astrovirus, enteric viruses, teschovirus

Poster Abstracts

Viral and Viral Diseases
OTHERS

PO-PF3-085

The detection and prevalence of ungulate tetraparvovirus 3 (porcine parvovirus 2) in European porcine samples.

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Introduction: The *Parvoviridae* is host to non pathogenetic and acutely pathogenic viruses across many host species. The novel parvovirus ungulate tetraparvovirus 3 (porcine parvovirus 2, PPV2) was detected in pig sera in Myanmar 2001. Subsequent studies have detected the virus in other countries. In south-eastern China, researchers isolated PPV2 from pigs with clinical symptoms of “high fever disease”. It still remains unclear if there is a correlation between disease association and the presence of this novel virus. The detection and prevalence of PPV2 was conducted on historical diagnostic clinical pig samples from 1996 to 2012 (n=783).
Materials and Methods: Various sample types (n=783) were collected from across Europe; France (n=340), Northern Ireland (n=253), Republic of Ireland (n=53), Great Britain (n=114) and Belgium (n=23). These were tested for PPV2. Viral nucleic acids were extracted from sera and 10% w/v homogenised tissue and faecal samples. In house primers were applied to detect PPV2 by SYBR Green PCR and positive samples were confirmed on agarose gel before sequencing.

Results: The overall prevalence of PPV2 was 19.3% (151/783) and the virus was detected in all countries. PPV2 was represented in most samples types; abortion fluid 1% (1/98), abortion tissue 0% (0/31), sera 6.1% 12/196, nasal swabs 2% (1/52), lung 50% (21/42), heart 13% (1/8), faeces 7% (2/28), colon and small intestine 45% (9/20), tonsil 28% 5/13, spleen 39% (14/35) and lymph tissue 33% (73/219). The pig sample age was known for n=465 of the samples. No PPV2 was present in abortion tissue samples or pre-weaner pigs (0/78). The weaner age group was positive for PPV2 at 12% (14/122), and the fattener and finisher aged pigs were positive at 51% (51/101) and 68% (19/28) respectively.

Conclusion: PPV2 was found to be widespread in European pigs. Presence of the virus in a wide range of tissues suggests a systemic tropism. The age breakdown of PPV2 virus infection indicates post-weaning exposure. This suggests that the majority of sows have been exposed previously and are providing protection to the piglets through maternal antibodies. There was a very low prevalence of PPV2 virus in abortion related samples perhaps suggesting that no vertical transmission occurs.
A number of novel parvoviruses have recently been detected within the pig population. The role in disease is unclear, they may cause disease outright, they could cause subclinical production losses and they could function as co-factors in poly-microbial conditions. In addition they all have the ability to mutate to more pathogenetic forms. As such continuous surveillance and research is important to determine the true role of these viruses in pig health.

Disclosure of Interest: None Declared

Keywords: porcine parvovirus 2, ungulate tetraparvovirus 3

Viral and Viral Diseases
OTHERS

PO-PF3-109

Senecavirus A outbreak affecting piglets and sows in Southern Brazil

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Introduction: Senecavirus A (SVA), is an emerging *picornavirus* that has been associated with outbreaks of vesicular disease and neonatal mortality in swine. Vesicular disease with unknown etiology and clinically indistinguishable of other vesicular infections have been reported in U.S, Canada and recently in Brazil. Here, we present an outbreak investigation conducted in swine herds showing an increased neonatal mortality and vesicular disease that have been associated to SVA.
Materials and Methods: Herds A and B are small farrow-to-finish (518 and 843 heads, respectively) research farms that belong to the Brazilian Agricultural Research Corporation (Embrapa Swine and Poultry) in Santa Catarina, Brazil. Herd A is located at 0.2Km from herd B. Both herds are vaccinated for PCV2, atrophic rhinitis, *E. coli*, PPV, leptospirosis, erysipelas and M. hyo. Brazil is free of PRRSV, and the farms are located in a Brazilian state free of CSF, PRV and FMD without vaccination. Clinical samples collected from herd A and B included nasal swabs, vesicular fluids and skin of coronary band. For herd B, three piglets were necropsied and tissues samples were collected for histopathology and for RT-PCR for SVA detection, targeting the VP1-VP3 region.
Results: Clinical signs were first seen in Herd A on October 30th, 2015 and remained until November 8th. On Herd B, clinical signs started on November 3rd. On herd A, five gilts, 28 sows (25 in the gestation and three in the farrowing building) and two boars were affected. On herd B, two sows and piglets with 3 to 5 days-old were affected. Sows from both herds presented vesicles mainly on the snout. Gilts and boars showed lameness and ulcerative lesions on coronary bands. Fever was not observed. Piglets were lethargic and had a watery diarrhea. The mortality rate in piglets increased from 11 to 34%. At necropsy, the piglets’s stomach was empty. It was observed enlargement and edema of inguinal lymph nodes, ascites, severe edema of mesocolon and severe necrosis of coronary band. Microscopic lesions were characterized by necrotic epidermitis and dermatitis of coronary band, mild enteritis with villus degeneration on small intestine and marked mesocolon edema. Senecavirus A was detected by RT-PCR in vesicular fluids of sows, skin of coronary band of gilts; and in intestine, tonsils and coronary band of piglets.
Conclusion: The concomitant ocurrence of vesicles in adult pigs and watery diarrhea with increased mortality in piglets was associated with SVA. Affected adult pigs recovered completely in 10-15 days. As SVA is clinically and economically important due to its resemblance with vesicular diseases, the diagnosis tools are essential.

Disclosure of Interest: None Declared

Keywords: Diarrhea, Senecavirus A, Vesicular disease

Viral and Viral Diseases
OTHERS

PO-PF3-066

Prevalence of the novel orthoreovirus 3 (MRV3) in diarrhea cases in US pigs in 2015

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Introduction: A novel mammalian orthoreovirus 3 (MRV3) has been discovered in cases of severe diarrhea at the beginning of the PEDV epidemic in 2013 in US field cases. It has been suggested that not PEDV alone but the combination of MRV3 and PEDV has caused the severe clinical signs seen in North American pigs.
Materials and Methods: In order to investigate the prevalence of MRV3, 277 randomly collected fecal samples from pigs with a history of diarrhea submitted to the Iowa State University Veterinary Diagnostic Laboratory from April to May 2015 were used. The samples originated from 41 farms and 1-to-34 pigs on each farm with an age range from neonatal to adult. All samples were tested with a real-time RT-PCR designed to target the S1 gene of MRV3. As an internal control, a portion of the samples were tested with a conventional MRV3 RT-PCR targeting also the S1 gene but resulting in a larger PCR product compared to the real-time PCR. A positive control, courtesy of Dr Subbiah Elankumaran, Virginia Tech, Blacksburg, USA, was included in all runs. In addition to MRV3 testing, all samples were also tested for PEDV RNA and PDCoV RNA by real-time RT-PCRs.
Results: Low levels of MRV3 RNA were detected in 5.7% (16/277) of the investigated samples from 19.5% (8/41) of the farms corresponding to 1 to 7 samples on each farm by real-time RT-PCR and two samples were confirmed by the conventional RT-PCR assay. Interestingly, in 1/8 MRV positive farms PEDV was detected but the remaining 7/8 MRV3 positive farms were negative for PEDV and PDCoV. The overall prevalence of PEDV in the investigated sample set was 10.5% (29/277) and corresponded to 22% (9/41) of the farms. The overall prevalence of PDCoV was 0.4% (1/277) and the positive sample was identified in a PEDV positive farm.
Conclusion: Our results indicate that the prevalence of MRV3 in the Midwest during 2015 is comparable with that of other enteric pathogens such as PEDV. Since 2015, the prevalence of PEDV is continuously decreasing indicating that herds are becoming immune to this virus. Our findings suggest that a similar scenario may also apply to MRV3, however, prevalence studies for MRV3 during 2013, when the virus was first recognized, and serological studies during 2013-2015 need to be conducted to further confirm this.

Disclosure of Interest: None Declared

Keywords: Diarrhea, Orthoreovirus-3, USA

Viral and Viral Diseases
OTHERS

PO-PF3-108

Comparative genomic characterization of pseudorabies viruses associated with pseudorabies outbreak in china

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Introduction: Pseudorabies virus (PRV) causes the globally notifiable Aujeszky's disease (AD) or pseudorabies (PR) in pigs. It causes significant economic losses to the pig husbandry. Since the live or killed vaccine had been widely used in China in 1970s, porcine pseudorabies was well controlled in China and worldwide. However, since late 2011, the outbreak of PR was reported in more than 20 provinces in China, causing the death of one million of pig. The Bartha-K61 vaccine does not provide effective protection against the novel PRV infection. To illustrate the genomic characterization of the highly pathogenic PRV in China. We sequenced and compared two novel viruses and two pevious PRV Fa and Ea strains.
Materials and Methods: PRV HNX and HNB strains were isolated from different Bartha-K61 vaccinated pig farms in 2012 in China. PRV Fa and Ea strains that isolated in 1962 and 1990 in China were kindly provided by Dr Fang Liurong, Huazhong Agricultural University, China.
The genome sequences of PRV HNX, HNB and Fa strains were determined by next-generation sequencing (NGS) technology. The genome sequences of PRV Ea strain was determined by Sanger sequencing using universal primers. Phylogenetic trees and molecular evolutionary analyses were determined by MEGA 5.05 software package.
Results: The PRV HNX, HNB, Fa and Ea strains had a genome 142,294, 142,255, 141,930 and 142334 nucleotides in length, and 73.56%, 73.61%, 73.70% and 73.60% G+C content, respectively. Homology analysis genomic sequences between HNX, HNB, Fa and Ea strains with Bartha strain were 90.1%, 90.6%, 90.7% and 90.3% respectively. The HNX, HNB strains showed 96.6% to 97.1% with Fa, Ea strains. Phylogenetic analysis based on the complete genome sequences revealed that the PRV HNX, HNB, Fa and Ea strains clustered to a relatively Chinese PRV strains group of the tree, and distant from non-Chinese PRV strains. However, the HNX and HNB strains were clustered to different subgroup with Fa and Ea strains. Analysis on the virulence associated gene and the immune associated gene sequences between the novel PRV strains and the previous PRV strains revealed that the virulence associated genes had slightly changes and clustered to the same subgroup, and the immune associated genes had mutations and clustered to the different subgroup.
Conclusion: The study indicate that the Chinese strains have been clustered different groups with non-Chinese PRV strains before using to be vaccinated with the Bartha-K61 vaccine. The novel PRV strains have been clustered different subgroups with previous PRV strains in China. The PR outbreak in many vaccinated farms in China may have related to mutations of immune associated genes but not virulence associated genes.

Disclosure of Interest: None Declared

Keywords: China, genomic characterization, Pseudorabies virus

Poster Abstracts

Viral and Viral Diseases
OTHERS

PO-PF3-048

Mass-exposure and tentative elimination of Senecavirus A in a Brazilian sow farm

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Introduction: Senecavirus A (SVA) is an emerging pathogen first reported recently in Brazil, causing vesicular disease in sows and finishing pigs. There is still lack of information regarding the pathogenicity and dynamics of infection. The objective of this abstract is to report the previous results of a tentative protocol of Senecavirus A elimination.
Materials and Methods: The tentative protocol was conducted in a sow farm, where vesicular lesions in nostrils and snouts predominantly, of sows (approximately 1-5% incidence), late nursery piglets (15%) and finishing pigs (2-3%) were observed. No other related clinical sign or production loss was observed. After 16 weeks of the onset of infection, a mass exposure was conducted with herd closure and spraying fresh oral fluids (OF) as an inoculum in the nostrils of every female. The OF samples were collected in pens with animals showing acute vesicular disease, and all sows and replacement gilts received at least two sprays, during weeks 1 and 2 of protocol. In week 0, no more offsite replacement gilts were brought into the sow farm, and between weeks 3 and 7 all piglets were weaned offsite, and so the herd was stable. During this period, a washing and disinfection process of the empty nursery facilities was conducted in three rounds, utilizing three different disinfectants, and finalizing with full drying, fumigation with formhoaldeid, followed by the return of onsite weaning.
Results: Until the date of submission of this abstract, the laboratory results of OF PCR and serology sampled during the protocol were not available yet. The clinical evaluation of the herd showed an increase in vesicular lesions in sow herd (~10%) after the first round of exposure, and on week 3 it returned to a baseline of 2-4% of prevalence. The first piglets being weaned in nursery started showing vesicular lesions in the nostrils and snouts on the second week (week 9), incidence of 10%.
Conclusion: The incidence of lesions in sows stabilized in 2-4% since the prime-infection and later after the mass exposure. Also, it was expected the absence of lesions in weaned piglets, due to the colostrum protection. The observation of vesicular lesions on the first weaned piglets onsite after the mass exposure of sows and herd closure may suggest that there was low success rate of the protocol. From the diagnostics and to the beginning of the exposure there was a 16 weeks period, when the dynamics of infection became endemic, and it probably interfered on the seroconversion of the whole sow herd, and consequently on the antibody transference to piglets. Further test results are necessary to learn more about the viral excretion of sows and piglets after the mass exposure and tentative elimination.

Disclosure of Interest: None Declared

Keywords: endemic infection, Herd closure, Vesicular disease

Viral and Viral Diseases
OTHERS

PO-PCO1-002

A novel diagnostic platform for in situ detection and subtyping of Rotaviruses and Influenza A in pigs

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Introduction: *In situ* hybridization (ISH) is a nucleic acid-based method that allows the detection of a particular RNA or DNA sequence within the tissue sections. A novel ISH RNA-based chromogenic technique (RNAScope) describes single-molecule visualization through the use of hybridization-based signal amplification system. These characteristics make this platform a promising diagnostic approach, especially by improving sensitivities issues faced by classical ISH techniques. The objective of this study was to evaluate the use RNA-ISH technique in a duplex assay for simultaneously detection Rotaviruses Groups A (RVA), B (RVB) and C (RVC), and Influenza A H1 and H3 genes.
Materials and Methods: Probes targeting specific genomic regions of the RVA, RVB and RVC (VP6 gene), and Influenza A (H1 and H3 genes) were developed based on validated PCR primers currently used at the veterinary diagnostic laboratory in University of Minnesota (MNVDL). Formalin-fixed paraffin embedded tissues were selected based on PCR results. Duplex assays were development with probes for Rotavirus group A and B, Rotavirus A and C, and Influenza H1 and H3. Hybridization signal was detected as green and red colorimetric staining followed by counterstaining with hematoxylin.
Results: Rotaviruses. A total of 30 samples of small intestines were evaluated. Four samples were positive by PCR for the three Rotavirus group with Ct ranged from 22 to 27 for RVA, from 27 to 30 for RVB and from 23 to 28 for RVC. One sample was positive for RVB (Ct 30) and RVC (Ct 23). Two samples were positive for RVA (Ct 20 and 22). Twenty-three samples were positive for RVA and RVC with Ct ranged from 16 to 28 for RVA and from 21 to 35 for RVC. Twelve samples negative by PCR for all three rotaviruses groups were used as controls.

Influenza A. A total of 16 lung tissues were evaluated. Eight were positive for both subtype H1 and H3 by PCR with CT values ranged from 15 to 33. Four were positive for H1 and four positives for H3. Five samples known as negative for Influenza A were used as controls.
There were no cross-reactions among the probes. ISH-RNA was able detected Ct value up to 31 in the intestinal epithelium for Rotavirus and up to 30 in the respiratory epithelium for Influenza A.
Conclusion: The designed probes were successfully able to differentially detected and *in situ* subtyped RVA, RVB, RVC and Influenza A H1 and H3. The lack of non-specific staining in the negative controls demonstrated the 100% specificity. In the samples evaluated, ISH-RNA sensitivity was higher than usually has been reported by IHC. The amplification steps applied in this platform may be critical for improving the ISH sensitivity.

Disclosure of Interest: None Declared

Keywords: Diagnostic, In situ hybridization, Rotaviruses, Influenza, subtyping

Viral and Viral Diseases
OTHERS

PO-PF3-182

Shedding patterns detected by PCR in sows and piglets of Senecavirus A from an infected U.S. swine breeding herd

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Introduction: The objective is to determine patterns of viral shedding, detected by PCR, from dams and their piglets for Senecavirus A (SVA) in serum, feces and oropharyngeal swabs for a 6 week time period post clinical onset.
Materials and Methods: A 4,000 head breeding herd developed clinical signs consistent with vesicular disease in sows and significant increases in neonatal mortality associated with the presence of SVA. Clinical signs were prominent in farrowing rooms with piglets less than 7 days of age. Clinically affected litters ranged from 30-40% of the litters in these rooms. Clinically affected litters were defined as the dam showing clinical signs and/or the litter having high neonatal mortality. In order to determine viral shedding and viremia, 11 affected and 11 unaffected dams and piglets were selected. For each affected litter, the dam and 2 piglets were tagged and sampled. For every unaffected litter, the dam and 1 piglet was tagged and sampled. For each sow and piglet tagged, serum, an oropharyngeal (tonsil) swab and a rectal swab were obtained. Samples were collected weekly for a period of 6 weeks and were tested by qPCR for SVA at the ISU VDL. A Ct of < 38 was used as the cutoff for calling a sample positive.
Results: PCR results after the first week of the clinical outbreak demonstrated the prevalence of PCR positives were fairly low in sows and pigs (10-30%) and only slightly higher when comparing clinically affected dams and piglets to non-clinically affected. Tonsil and rectal samplings were positive in about half the dams that were clinically affected, while only about 1/4 were positive in non-clinically affected dams. Piglet tonsil samples were positive in about 1/3 of the samples collected, regardless if they were in clinically affected litters or not. Rectal samples were positive in about half of the non-clinically affected piglets, where they were positive in only 1/3 of piglets. Most serum positive piglets were also positive in tonsil and fecal samples. The dam results were more variable in terms sample type. Virus was detectable in serum for a period of 3 weeks post outbreak in both sows and their piglets, demonstrating a relatively short viremia. A high frequency of shedding was detected in tonsil and rectal swabs out to 4 weeks post outbreak.
Conclusion: During the initial outbreak, PCR positive samples for SVA could be found in both clinically and non-clinically affected dams and their piglets. There appeared to be a slightly higher prevalence in clinically infected dams and their litters. Based on these PCR results, SVA viremia lasts for a period of 3 week in piglets and sows and virus is shed for 4 weeks.

Disclosure of Interest: C. Rademacher Conflict with: Swine Health Information Center, D. Linhares Conflict with: Swine Health Information Center, P. Pineyro Conflict with: Swine Health Information Center, P. Canning Conflict with: Swine Health Information Center, D. Holtkamp Conflict with: Swine Health Information Center, L. Karriker Conflict with: Swine Health Information Center

Keywords: Senecavirus A

Viral and Viral Diseases
OTHERS

PO-PF3-113

Porcine parvoviruses are prevalent in commercial pig farms in Poland

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Introduction: Parvoviruses have been circulating in the global pig population for some time. So far, six porcine parvoviruses have been described in pigs including the classical PPV1 commonly associated with reproductive failure in breeding herds and newly recognized PPV2, PPV3, PPV4, PPV5 and PPV6. An association of porcine circovirus type 2 (PCV2) and PPV1 (also known as the classical PPV) or the PPV2 with porcine circovirus associated disease (PCVAD) has been established. The association of the remaining recently described parvoviruses with clinical disease in pigs remains unknown. The objective of this study was to investigate the prevalence rates of PCV2, classical PPV1 and recently recognized PPV2, PPV3, PPV4 and PPV5 in serum samples from pigs in Poland.
Materials and Methods: Serum samples (n=159) were collected from pigs of different age groups (2, 5, 9, 13 and 17 weeks old) in seven commercial PRRSV positive pig farms in Poland as part of a PRRSV surveillance study. Nucleic acids were extracted from the serum samples using an automated system. All samples were tested for presence of PCV2, PPV1, PPV2, PPV3, PPV4 and PPV5 by real-time PCR assays.
Results: PCV2 was detected in 3.1% (5/159) of the serum samples corresponding to 3/7 farms. PPV1 was detected in 5.0% (8/159) of the serum samples in 3/7 farms. PPV2 was detected in 6.3% (10/159) of the samples in 4/7 farms. PPV3 was detected in 7.5% (12/159) samples in 4/7 farms. PPV4 was detected in 6.9% (11/159) of the samples in 5/7 of the farms. Finally, PPV5 was detected in 5.0% (8/159) of the samples in 4/7 farms. All seven farms had at least two different parvoviruses co-circulating, and four different parvoviruses were identified in 2/7 farms. Overall, 27.0% (43/159) of the samples were positive for at least one of the tested pathogens and among those samples, 18.6% (8/43) were coinfected with two or more pathogens. The majority of positive samples (86%, 37/43) were found in pigs between 9 and 13 weeks of age, which corresponded to only 47% of tested pigs (75/159).
Conclusion: A high overall prevalence of PPVs was identified in serum samples obtained from 9 to 13 weeks old pigs in Poland. Prevalence of PPV4 and PPV5 was relatively higher than previously described in other geographic regions.

Disclosure of Interest: None Declared

Keywords: Poland, Porcine circovirus type 2, Porcine parvovirus

Poster Abstracts

Viral and Viral Diseases
OTHERS

PO-PF3-056

Identification and genetic comparison of VP1 gene of Seneca Valley Virus in Brazil

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Introduction: Vesicular disease has been described recently in Brazil and was associated to Seneca Valley virus (SVV). The clinical presentations were characterized by vesicular lesions in sows and acute losses of neonatal piglets. SVV has been identified in swine herds from United States, Canada, Australia, Italy, China and New Zealand. Few reports in Brazil characterized the virus circulating in outbreaks in the country and performed the phylogenetic analysis to better understand the epidemiology of the virus. Based on that, the objective of this study was to identify the SVV circulating in Brazil and perform the phylogenetic comparison with other virus from Brazil, US and China.
Materials and Methods: Thirteen samples: vesicular fluid (n=1), lungs (n=5), lymph nodes + spleen (n=7), submitted to our laboratory from clinical vesicular disease had the total RNA extracted using RNeasy™ kits (Qiagen) The RNA (10 µl) was used as the template in a one-step RT-PCR with the primers SVV-1C556F (5'-TCGGTTTACTCCGCTGATGGTTGG-3') and SVV-2A22R (5'-AGGACCAGGATTGGTCTCGATATC-3')destined from SVV VP1 gene and using the following cycling conditions: 30 min at 42 °C, 5 min at 94 °C, and then 35 cycles of 1 min at 94 °C, 1 min at 55 °C and 1.5 min at 72 °C, followed by 5 min at 72 °C and held at 4 °C. PCR products were analyzed by electrophoresis using 1.5% agarose gels, ran at 120 Volts for 40 min and visualized using a gel documentation system. The positive samples had the DNA sequencing performed and compared with other SVV sequence available at online public database. The RNA samples were also tested for vesicular stomatitis.
Results: The vesicular fluid sample was PCR positive for SVV and all samples were PCR negative for vesicular stomatitis. The SVV positive sample shared 99% of identity with the Brazilian SVV strains: SVV/BRA/GO3/2015, SVV/BRA/MG2/2015 and SVV/BRA/MG1/2015 and 98% of identity with strains BRA/UEL-SVV-A1/15 and BRA/UEL-SVV-B2/15. To North American SVV strain USA/IA40380/2015 shared 97% of identity and to USA/SD41901/2015 and USA/IA46008/201 shared 96% of identity. And to a Chinese strain CH-01-2015 was observed 96% of identity.
Conclusion: A considerable sequence identity between our strain and the other Brazilian strains were found. Evidence of the introduction of the SVV and the distribution of this virus in the country still unclear, however, further studies are being made to better understand and identify the distribution of SVV in Brazil. The characterization of this virus could contribute to future control strategies, especially to avoid positive replacement animals.

Disclosure of Interest: None Declared

Keywords: Brazil, SVV, Vesicular disease

Viral and Viral Diseases
OTHERS

PO-PF3-002

Whole Genome Sequencing on the Identification of Pathogens Involved with High mortality in Piglets

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Introduction: Idiopathic vesicular disease (IVD) is a sporadic condition that affects swine herds worldwide. At the end of 2014, swine herds across Brazil showed outbreaks similar to IVD causing high mortality and the presence of vesicles in piglets, and erosions on the snouts and coronary bands of sows. Samples of infected animals were shipped to Lanagro (Agriculture Ministry Official Laboratory) and tested for vesicular diseases including foot and mouth disease with negative result. Some studies have identified the association of *Seneca valley virus (SSV)* in pigs affected with IVD. Therefore, a metagenomic study in samples from brain, kidney and vesicles fluid and epithelium (VFE) of infected animals was carried out as a diagnostic tool to identify possible pathogens involved with this condition using two approaches.
Materials and Methods: Approximately 0.05 g of tissue samples from brain, kidney and VFE of infected pigs were submitted to RNA and DNA extraction at the Embrapa Swine and Poultry NB3 Lab. Approximately 2ug of RNA was reverse transcripted using high capacity cDNA kit and random primers. DNA and cDNA from individual tissue samples were pooled together from 3 animals for each tissue, and shipped to PathGEN Dx Pte. (Singapore). The first pathogen screening was conducted with the PathGEN® PathChip, which contains 50,000 viruses and 20,000 bacteria, developed by Affymetrix®. Following the identification of the possible infectious agents, the DNA and cDNA samples were enriched by RT-PCR using PathGEN primers, and the sequencing library was created using standard protocol. Raw pair-end reads were generated with the Illumina MiSeq (2x250bp). Following sequencing the *Seqyclean* program was used to clean the data, removing adapters, contaminants, short reads < 70bp, and reads with Phred < 20. All reads were mapped using BWA program against two known Seneca Virus sequences and assembled using Newbler.
Results: Using the PathGEN® chip we identified partial sequences of human rhinovirus (HRV) only in Brain samples. The SVV has a structural similarity up to 40% with HRV which can be responsible for the results. Using the MiSeq technology for sequencing allowed us to build partial fragments of the SVV in two samples (brain and kidney), and the complete sequencing in VFE of infected animals.
Conclusion: SVV sequence was identified and associated with IVD observed in Brazil since 2014. SSV was found in three different tissues: brain, kidney and VFE when sequencing approach was used, and only in the brain using PathChip. The whole SVV sequence assembled in this study shows some variations when compared to the ones published.

Disclosure of Interest: None Declared

Keywords: Metagenomic Sequencing, PathGEN®, Seneca Virus

Viral and Viral Diseases
OTHERS

PO-PF3-160

Involvement of porcine astrovirus in an enteric episode in piglets in Italy

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Introduction: Astroviruses are emerging viruses in the Family Astroviridae that infect a wide range of mammalian and avian species. They are non-enveloped viruses with a single-stranded positive sense RNA genome. They can be found in the intestines and several other organs in diseased and healthy animals. Porcine astroviruses (PAstVs) belong to the Mamastrovirus genus and are distributed worldwide. Five lineages are recognised, possibly reflecting different species of origin, interspecies transmission and recombination events, these latter having occurred even with human strains. Here we report a diagnostic case of acute gastroenteritis in piglets in Italy with the involvement of PAstVs.
Materials and Methods: In November 2015 an acute episode of gastroenteritis was observed in pig farm located in North east Italy, Treviso province. The farm is organized in an open cycle of about 460 sows. Piglets during the first phases of post weaning presented diarrhea, reduction of food intake, high morbidity and low mortality. Fecal samples collected from diseased piglets and one piglet were submitted to the diagnostic veterinary laboratory. Bacteriology tests were performed on intestine, liver and faeces. Faeces were analysed by electron microscopy (EM) and by a pan mamastrovirus RT-PCR. Sequence and phylogenetic analysis was performed on the polymerase gene obtained from PCR positive samples.
Results: The necropsy on dead piglet showed a discrete nutrition status with severe catarrhal enteritis with foam and lose of tone of the intestinal tract. Peritonitis, edema of colon and hyperemia of pyloric region of gut were also observed. Presence of Escherichia Coli not belonging to relevant serogroups or serotypes in intestine and faeces was detected. Presence of Salmonella and Clostridium perfringes was excluded. Faeces were positive for astrovirus by EM and RT-PCR. Sequence BLAST analysis of the 288 bp of polymerase gene revealed the highest homology with PAstV 2 from South Korea and with Porcine Mamastrovirus 3 from China. The detected astrovirus clusters with a lower homology with other PAstV 2 from US and, interestingly with a deer astrovirus isolated in Denmark.
Conclusion: The present case report indicates that PAstVs may be involved in enteric disorders in piglets. Genetic data highlight the complexity of PAstVs classification and may suggest a unique ability to cross the species barriers. However still fragmentary data are available on the role of PAstVs in the multifactorial enteric disorders of pigs. Better surveillance and diagnosis in the field of enteric disorders in pigs may clarify the epidemiology and taxonomical classification of PAstVs.

Disclosure of Interest: None Declared

Keywords: astrovirus, Enteric disease

Viral and Viral Diseases
OTHERS

PO-PF3-157

Isolation and Evoluton of a new emergent Porcine Deltacoronavirus stain in China in 2015

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Introduction: Porcine deltacoronavirus(PDCov),a coronavirus, develop the pathological symtoms similar to PEDV and TGEV such as severe watery diarrhea and high mortality in piglets,but the mortality rates were lower. This disease was initially reported in 2012 in HongKong. PDCov caused outbreak in farms 17 US states on December 2014 and it was also reported in Korea.PDCov is an enveloped,single-stranded,positive sense RNA virus in the family coronaviridae, subfamily coronavirinae. PDCov has a genome of approximately 25.4 Kb and includes six common coronaviral genes:5'untranslated regin(UTR)-ORF1a-ORF1b-S-E-M-N-3'UTR.
In present study, a reverse transcription PCR(RT-PCR) method was developed to detect PDCov and samples from a severe diarrhea outbreak in farms in JiangXi province. PDCov N gene sequecing,phylogenisis,viral isolation and future studies were conducted and a new emergent PDCov isolate was reported and named Ch-A strain.
Materials and Methods: Small intestine samples of piglets with diarrhea from JiangXi province were colled to run RT-PCR by primers targeting N gene of PDCov while were also detected by PCR with same samples. Then PDCov positive PCR products were cloned and sequenced to analyse the evolution of this strain through biologic software Mega6.0.
Results: The sample showed positive in RT-PCR of PDCov N-gene target but negative of TGEV,PEDV or RTV target. Compared PDCov Ch-A N gene with that of USA and Korea strains, it shared 99.0%>99.8% aminoacid sequence identity with US strain and 98.5%>99.0% aminoacid sequence identity with Korea strain respectively. Phylogenetic analysis of N gene of PDCov strains showed that CH-A formed a new independent branch. This strain was propergated primary swine testicular cell cultures of day-old pige and the virus was successfully propagated up to 10 pssages.
Conclusion: A new emergent PDCov CH-A strain was comfirmed and isolated in China in 2015. The virus were successfully propagated in cell culture.

Disclosure of Interest: None Declared

Keywords: PDCoV;Isolation;Evolution

Poster Abstracts

Viral and Viral Diseases
OTHERS

PO-PF3-158

Demonstration of Senecavirus A protective immunity in a pig model

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Introduction: Although idiopathic vesicular disease (IVD) is rarely diagnosed in US swine, in 2015 there were over 100 cases identified throughout the country. Frequently, Senecavirus A (SVA) has been associated with IVD and is presumed to be the etiologic agent. In recent studies we have shown SVA to induce a vesicular disease in nursery and finishing age pigs. This paper describes initial efforts demonstrating SVA protective immunity.
Materials and Methods: Two litters of pigs (n=10, n=6) were used at 1 and 7 days-of-age, respectively (time = D0). On D0, one-half of each litter was weaned into a separate room (Group A). Each remaining half (Group B) was kept with their dam until D14, and each pig and both sows received an intranasal inoculation of the 15-41901SD SVA isolate ($5x10^7$ pfu/pig). Serum and swabs samples were collected from Group B pigs at D4, 10 and 20. On D45, all piglets in both groups received an intranasal inoculation of the same viral stock at the same dose. Clinical observations, serum and swab samples were collected at D45, 47, 49, 52, and 54 at which time all pigs were euthanized. Sows were euthanized on D14. All serum and swab samples were tested for SVA by PCR.
Results: In Group B pigs on D4 SVA was detected in 8/8, 7/8, and 8/8 serum, fecal and oral swab samples, respectively. On D10, SVA was detected in 3/8, 6/8, and 8/8 serum, fecal and oral swab samples, respectively. No SVA was detected in any sample on D20. No cutaneous lesions were observed in Group B. In sows, a PCR positive serum and fecal swab were detected in one sow on D10, all other samples from either sow (D4, D10, D14) were negative. On D8 a sow developed a vesicle on her snout, no other cutaneous lesions were observed in either sow. Following challenge at D45, all Group A pigs were PCR positive in one or more samples on each day. All Group A pigs developed one or more cutaneous lesions located on coronary band, interdigital space, or tongue by D54. SVA was detected in vesicular lesions. In contrast, none of the pigs in Group B were PCR positive, nor developed any cutaneous lesions following second SVA challenge.
Conclusion: Prior SVA infection of neonatal pigs induced a homologous protective immunity when tested 45 days later demonstrating young pigs can be used as a model to study the SVA immune response.

Disclosure of Interest: None Declared

Keywords: Immunity, nursery pigs, Senecavirus A

Viral and Viral Diseases
OTHERS

PO-PF3-047

Clinical experiences with neonatal mortality and vesicular disease associated with Senecavirus A in Brazil and USA pig farms

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Introduction: Vesicular disease (VD) was reproduced with Senecavirus A (SVA) in 9 and 25 week old pigs, confirming that SVA causes VD in pigs (ISU and USDA, 2015). Between Sept 2014 and Dec 2015 a large incidence of VD associated with SVA was reported in Brazil. Likewise, between July-Dec 2015 the incidence of SVA in the USA pig industry increased significantly. Moreover, authors of this study reported the epidemic transient neonatal losses (ETNL) syndrome affecting piglets in Brazil and USA. In most cases, ETNL was associated with minor/mild VD. The objective of this case report was to describe clinical presentation and diagnostic investigation of cases of ETNL and VD associated with SVA infection in Brazil and USA herds.
Materials and Methods: We followed 4 SVA outbreaks: Farm A (8000 breed to finish), Farm B (4000 breed to wean) and Farms C and D (growing pigs). Clinical features and differential diagnosis associated with ETNL and VD in enrolled farms were described.
Results: *Farms A and B.* Sudden increase in mortality rate on piglets of 0-4 days of age was reported. Farm A mortality was 72% in that age group and returned to baseline after 7 days. Farm B mortality was 38% and returned to baseline 5 days. In both cases there were affected and non affected litters. Within affected litters, close to 100% pigs were affected. Necropsy of 40 affected pigs per farm evaluated gross and histopathology lesions. No significant lesions were noticed. About 85% of piglets had stomach full of milk, indicating that starvation did not cause death. On farm A, 20% of sows developed minor VD in the foot (coronary bands, foot pad and/or interdigital area) and 2% of sows had snout vesicles - all VD healed within 2 weeks. Farm A finishing pigs developed lameness followed by VD in 30% of pigs, starting 1 week after ETNL onset and recovering in 2 weeks. No VD was observed in farm B. Lameness was reported in farms C and D. Farm C had a high incidence (80%) of pigs with lameness followed by VD lesions in foot and/or snouts within 10 days. Lesions had mild severity and recovered within 14 days. No VD was observed after the first wave. On Farm D, 20% pigs developed VD in week 1, and about 3-5% incidence per week in the next 12 weeks were reported.
A comprehensive diagnostic investigation was performed and included infectious and non-infectious etiologies. Presence of other viruses causing vesicular disease including FMDv was ruled out. SVA infection was initially associated with the disease through the identification of viral RNA by PCR. In addition, SVA was identified within the tissues by *in situ* hybridization.
Conclusion: SVA caused lameness and VD in pigs and was associated with ETNL.

Disclosure of Interest: None Declared

Keywords: ETNL, Senecavirus A, Vesicular disease

Viral and Viral Diseases
OTHERS

PO-PF3-167

PHYLOGENETIC ANALYSIS OF TORQUE TENO SUS VIRUS SPECIES FROM CASES OF POSTWEANING MULTISYSTEMIC WASTING SYNDROME IN MEXICO.

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Introduction: Torque teno sus virus (TTSuV) is a circular single-stranded, negative sense DNA virus that belongs to *Anelloviridae* family. TTSuV comprises two genres: *Iotatorquerivirus* with two species (TTSuV1a and TTSuV1b), and *Kappatorquevirus* with one species TTSuVk2). TTSuV are ubiquitous in domestic and wild porcine population and co-infection among species is regarded frequent. Furthermore, a high rate of co-infection with PCV2 has been reported, predominantly from cases of PMWS.
Materials and Methods: To perform a phylogenetic analysis of TTSuV from cases of PMWS, 44 paraffin-embedded lymphoid tissues fulfilling international diagnostic criteria of PMWS and 11 lymph nodes from healthy age-matched piglets were selected to amplify the complete ORF2 of TTSuV1a and TTSuV1b by nested PCR. The amplified products were purified and sequence to construct phylogenetic trees with the Maximum Parsimony method. Statistic trust of the topology was assured with bootstrap values, 1000 repetitions. Bootstraps results over 70 (700) were considered highly similar.
Results: Four TTSuV1a and 15 TTSuV1b amplified products, were purified and sequenced. The complete ORF2 was obtained in all instances. At alignment with available sequences from Genbank, 219 nucleotides displayed lecture for 72 amino acids, revealing 13 exchanges, and 207 nucleotides with lecture for 68 amino acids and 22 exchanges for TTSuV1a and TTSuV1b, respectively. The topography of TTSuV1a phylogenetic tree showed that amplified sequences are specific of species 1a, having boostrap values of 99 with Chinese, American and Canadian sequences. For TTSuV1b, the topography depicted that the majority of sequences belong to species 1b, displaying boostrap values of 77 and 100 with Chinese and Canadian; respectively, but 4 Mexican sequences belong to species k2 since they display boostrap of 76 and 98 with Brazilian sequences which are reference strains of TTSuVk2. All Mexican sequences have lower boostrap with Spanish strains.
Conclusion: The present work has documented that the three species of TTSuV are present, sharing a higher similarity with Asian and American sequences, predominantly with the Chinese, but lower homology with European. The latter might explain dissimilar frequencies with European data since the Mexican TTSuV prevalence from cases of PMWS has been found significantly lower. Furthermore, our results have shown that existing Mexican TTSuV strains possess a considerable nucleotide variability, leading to several amino acids replacements. Those changes might be related to a different pathogenic potential.

Disclosure of Interest: None Declared

Keywords: TTSuV1/2, PCV2, PMWS Phylogenetic

Viral and Viral Diseases
OTHERS

PO-PF3-253

Discovery of Porcine Deltacoronavirus (PDCoV) in Taiwanese Pig farms

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Introduction: Porcine Deltacoronavirus (PDCoV) belongs to the fourth genus *Deltacoronavirus*, in the family *Coronaviridae,* in the order *Nidovirales*. It is an enveloped, positive sense, single-stranded Ribonucleic Acid (RNA) coronavirus with a genome size between 25.4 to 26.6Kb.
Characterized by clinical symptoms associated with Transmissible Gastroenteritis Coronavirus (TGEV) and Porcine Epidemic Diarrhoea Virus (PDEV) in neonatal piglets and sows. Field reports suggest that acute watery diarrhoea and vomiting is prevalent amongst swine infected with different PDCoV strains, occurring continuously in infected piglets and sporadically in sows. Since PDEV is the dominant enteric virus in Asia, there is a probability that PDCoV may have been overlooked due to their indistinguishable clinical signs. This could potentially cause decrease productivity to the pig farming industry in Taiwan.
Materials and Methods: Specimens were derived from previous clinical cases submitted to the National Pingtung University of Science and Technology (NPUST) Animal Disease and Diagnostic Centre from January to December 2015. These clinical cases were previously screened for TGEV and PEDV, in order to determine the etiological agent of disease. Each specimen was assigned a case identification number and stored in the Centre's database. A Real-time Reverse Transcription PCR assay using Primers and Probes designed specifically to detect for the presence of PDCoV was utilised.
Results: Of 795 specimens tested, 49 (6.16%) were tested PDCoV positive. Of 176 herds, 34 (19.21%) were tested positive. We detected the highest amount in the contents of Ileum and rectal swabs, 8.58% (23/268) and 5.56% (12/187) respectively. Followed by milk, 3.69% (11/278); oral fluids, 20% (2/10) and small intestine, 11.11% (1/9). The samples were further classified according to the swine age. Of 49 specimens, 21 (34.69%) were from suckling pigs, 13 (22.45%) nursing pigs, 3 (6.12%) growing pigs, 12 (24.49%) sows were tested PDCoV positive. Of the 49 PDCoV positive, 20 were co-infected with PEDV (40.82%). Suckling pigs were also noted to be susceptible to co-infection (12/21).
Conclusion: To our knowledge, this is the first study on PDCoV conducted in Taiwan. Our findings revealed that PDCoV is indeed present in Taiwan.

Disclosure of Interest: None Declared

Keywords: PDCoV, Porcine Deltacoronavirus

Poster Abstracts

Viral and Viral Diseases
OTHERS

PO-PF3-128

Identification and phylogentic analysis of novel parvoviruses, cyclovirus and stool-associated virus in tissue and faeces samples from pigs

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Introduction: The control of viral diseases is an ongoing battle for pig farmers to prevent the economic losses associated with mortality and morbidity. Horizon scanning and monitoring of emerging threats is key to this control. This explorative study aimed to identify emerging viruses circulating within Northern Ireland stocks. The viruses investigated were emerging porcine parvoviruses (PPV5 and PPV6, genus *Copiparvovirus*), porcine CyCVovirus (CyCV) and porcine stool-associated circular viruses (PoSCV). PPV1 is associated with reproductive failure in sows but in recent years a wide variety of parvoviruses have been identified, of unknown aetiology. CyCV and PoSCV are both circular single stranded DNA viruses, again of unknown disease association.
Materials and Methods: 121 porcine mesenteric lymph nodes (MLN) from veterinary submissions and 135 faeces samples from healthy slaughter age pig were collected in Northern Ireland. Primers were designed in-house using Primer3 software on multiple sequence alignments generated with Geneious. PCR was carried out using Sybr Green I according to standard protocols. Separate primers were designed to amplify overlapping regions of the virus genomes for sequencing and subsequent phylogenetic analysis. Phylogenetic trees were produced for each virus using Mega5.
Results: Positive samples were confirmed by melting curve analysis and by gel electrophoresis.
MLN samples; 10/121 (8.3%) were +ve for PPV5, 1/121 (0.8%) was +ve for PPV6 and 0/121 (0%) were +ve for CyCV and PoSCV. Faeces samples; 23/135 (17%) were +ve PoSCV.
Phylogenetic analysis was carried out on 5 PPV and 1 PPV6 capsid sequences. Resultant BLAST search and phylogenetic trees confirmed the sequences as PPV5 and PPV6. Similarly the analyses confirmed the PoSCV to belong to group 3.
Conclusion: Small DNA viruses are ubiquitous in pig herds. PPV5, PPV6 and PoSCV were detected in this study while CyCV was not. The significance of these novel viruses remains debatable in terms of their potential to cause disease. They may be non-pathogenic, they may be could function as co-factors in polymicrobial diseases or they could cause subclinical effects resulting in reduced performance. All these viruses have the potential to mutate into more virulent forms. Monitoring the prevalence and evolution of such emerging viruses and researching potential roles in pathogenicity are therefore important activities for the understanding of their impact on animal health. Advanced knowledge of novel viruses will benefit our preparedness in the event that they become problematic.

Disclosure of Interest: None Declared

Keywords: emerging , pigs, virus

Viral and Viral Diseases
OTHERS

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Evaluation of potency test against cattle, pig in order to revision of Foot-and-Mouth Disease vaccine national assay of South Korea

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Introduction: Foot-and-mouth disease (FMD) causes severe economical problems in livestock industry because of rapid spread and inducing low productivity. Thus, the administration of vaccines is a highly effective method for preventing FMD. In an effort to prevent and control FMD, Korea is currently implementing a vaccination policy. Despite all the hard work, Korea is suffering from recent FMD outbreaks of serotype O. FMD vaccines were evaluated characteristics, purity, sterility, mycoplasma contamination, preservative contents, pH, safety, potency test in order to revise Korean FMD vaccine standard assay.
Materials and Methods: The DOE high potency (≥6PD50) vaccines (O1 Manisa and O 3039 strain) were used in this study. Eighteen cattle of more than 3 months old, 27 conventional pigs and 24 SPF pigs of more than 8 weeks old were vaccinated intramuscularly (6 group of animal received the two times dose, a full dose, 1/2 dose, 1/4 dose, 1/10 dose, none of vaccine, respectively). All animals were screened sero-negative before vaccination. For the serological test, blood samples were collected with the 2, 3 and 4 weeks after vaccination. All the sera were tested in virus neutralization test and ELISA.
Results: In serological assay, mean VNT titres were dose-dependent that maens coefficient of correlation (r value) was higher in cattle and SPF pigs more than the conventional pigs. And all SN titres of three serotype were more than 1.42 log VNT (cut-off of vaccine company) in 21dpv. The results of correlation between animals were some relation to each other.
Conclusion: We evaluated potency of FMD vaccine about O1 Manisa and O 3039 strain in target animals. On the basis of our results, we may consider the antibody level of field animals and the present condition of FMD biosecurity in Korea. Further study is required to confirm the cut-off of FMD vaccine national assay standard based on field animal experiments.

Disclosure of Interest: None Declared

Keywords: FMD, potency test , Vaccine

Viral and Viral Diseases
OTHERS

PO-PF3-067

Discovery of a divergent lineage porcine pestivirus in piglets with congenital tremors

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Introduction: Congenital tremors is a disease of neonatal pigs characterized by action-related repetitive myoclonus. Despite early documentation (nearly 100 years ago) and worldwide distribution of the disease, the etiology of a majority of contemporary outbreaks remains a mystery but has been speculated to be an unidentified virus. This investigation describes the identification of a divergent lineage pestivirus in samples from piglets with congenital tremors and not unaffected cohorts.
Materials and Methods: Five unrelated farms located in the United States in the state of Iowa (n=4) and Illinois (n=1) reported clinical signs compatible with congenital tremor. Animal from Farm A included 15 piglets of which 11 were affected. Animals from Farm B included 5 affected piglets. Animals from Farm C included 4 affected piglets and 3 unaffected. Animals from farm D included 5 affected and 1 unaffected. Animals from Farm E included 7 affected and 6 unaffected piglets. Samples including serum, cerebrum, cerebellum, brainstem, and spinal cord were collected. Varied porcine tissues (serum, cerebrum, cerebellum, and/or spinal cord) from Farm A and Farm B were processed for MiSeq based sequencing through library generation using NextEra XT library preparation kit (Illumina) per the manufacturer's suggested protocol, with replacement of column elution (Qiagen, MinElute) in lieu of bead normalization. A quantitative one-step RT-PCR (RT-qPCR) was developed targeting the NS3 region of the divergent lineage pestivirus and was run on tissue and/or serum samples from Farm A, B, C, D and E.
Results: Through the use of next-generation sequence technology a divergent lineage virus originally most closely related to a Chinese bat pestivirus and now known to be more closely related to a recently reported porcine pestivirus was identified in samples from piglets with congenital tremors. The virus was detected by RT-qPCR in multiple sample types from affected piglets (31 out of 32) that originated from five unrelated farms and was not commonly detected in unaffected piglets (2 out of 14). Phylogenetic analysis of the NS3 and Npro amino acid sequences support classification of the virus identified herein as a member of the putative "atypical porcine pestivirus" species with 88.0% and 94.6% nucleotide and amino acid identity, respectively. This divergent lineage pestivirus virus is phylogenetically distinct from classical swine fever virus with an amino acid percent identity of the viral Npro ranging from 35.9 to 36.4%.
Conclusion: This report describes the identification of a divergent lineage porcine pestivirus in piglets with congenital tremor from five unrelated farms located in two different states in the United States.

Disclosure of Interest: None Declared

Keywords: action-related repetitive myoclonus, Congenital tremors, pestivirus

Viral and Viral Diseases
OTHERS

PO-PF3-256

Seroprevalence of Bovine Viral Diarrhoea Virus infections of different age groups in intensive pig farming

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Introduction: Infection caused by BVDV in pigs have been reported in countries with important pork industry such as China, Netherlands, the Brazil and others, which brings concern about the existence of accurate diagnosis tests, questions about the risk factors, epidemiology and the clinical form that BVDV infection may entail. Moreover, the antigenic similarity between BVDV and CSFV can generate cross-reactivity in diagnostic tests that challenging a fast and reliable diagnosis for both diseases. This research focused on assessing the prevalence of BVDV-1 and BVDV-2 infections in swine of different age groups from intensive pig farming.
Materials and Methods: A set a least of 30 swine serum samples from each different age groups (suckling piglets, nursery pigs, finishing hogs and sows) was collected in five different intensive pig farming, totalizing 607 samples. All farms were located in the northeastern region of São Paulo state. The virus neutralization assay was performed according to *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (OIE), using MDBK tissue culture cells. Both cytopathic strains Singer (BVDV-1) and VS253 (BVDV-2) were used in a 100 $TCID_{50}$ concentration. The final titer obtained by the geometric mean (GMT) of two tests.
Results: Out of 607 evaluated serum samples using BVDV-1 only one from a finishing hogs was positive (farm 2), consequently showing a prevalence of 3.33% in the age group, 0.81% within the entire herd and 0.16% of all samples. When used the BVDV-2, the farm 2 had 3.33% of positive samples in finisher hogs age group and 6.66% in the sows groups, presenting a herd prevalence of 2.44%. The farms 3 and 4 had 3.33% of prevalence in the sows group in each one. Whereas, in farm 5 were detected 6.66% of prevalence from sows group. Farm 3, 4 and farm 5 had within herd prevalence of 0.84%, 0.84% and 1.66% respectively. The results reveal that the infection of BVDV-2 is more frequently and the sows groups were affected, with prevalence of 4.0% of sows and 1.15% of all samples. To BVDV-1 the antibody GMT was 14.14214. Regarding to BVDV-2, all positive samples had GMT value of 10, except for a sample of farm 2 that had GMT of 20.
Conclusion: Despite low prevalence value found, the infection is predominantly present in sows, suggesting that this age group can be more susceptible because of longer exposure to infection and due inefficiency of biosecurity measures that allows interspecies transmission. Possibly, there is no transmission from pig to pig or, if any, is very limited. This study had financial support provided by grant 2014/13590-3, São Paulo Research Foundation (FAPESP).

Disclosure of Interest: None Declared

Keywords: BVDV, Epidemiology, virus neutralization

Poster Abstracts

Viral and Viral Diseases
OTHERS

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Description of anti-BVDV antibodies titers detected in swine serum by virus neutralization assay

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Introduction: The members of the *Pestivirus* genus are antigenically related, consequently cross-infections, such as BVDV infections in swine, are often reported. The virus neutralization assay (VN) is the standard diagnostic assay for BVDV infections in cattle according to the OIE. However, very little information is available in the scientific literature about the use of VN to detect and quantify antibodies anti-BVDV in swine serum. This study focused on describing our experience and obtained results in the detection of antibodies anti-BVDV in swine serum using the VN.
Materials and Methods: A set of 3,057 serum samples of swines from eight Brazilian states (Goiás, Mato Grosso do Sul, Mato Grosso, Paraná, Rio Grande do Sul, Santa Catarina, Rio Grande do Norte and São Paulo) were tested in duplicates using the VN to detect antibodies anti-BVDV. The assay was performed according to the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, using MDBK (*Madine Darby Bovine Kidney*) tissue culture cells. Both cytopathic strains Singer (BVDV-1) and VS253 (BVDV-2) were used in a 100 $TCID_{50}$ concentration. The positive samples were those in which occurred total neutralization of the 100 $TCID_{50}$ in a dilution higher than 1:10. The antibody titer considered was the reciprocal of the highest dilution in which there were total neutralization of the viral dose and the final titer obtained by the geometric mean of the two results.
Results: Out of all tested samples, 2.49% (76) were positive when using the BVDV-1 (Singer) as standard virus. Thus, 36.84% (28/76) of the animals had antibody titer 10; 25.0% (19/76) presented titer 14.14213562; 17.10% presented titer 20; 2.63% (2/76) had 28.28427125; 5.26% (4/76) had titer 40; 3.94% (3/76) had titer 80; 3.94% (3/76) had titer 160; 1.31% (1/76) had antibody titer of 320 and 3.94% (3/76) had titer 640.
When using the BVDV-2 (VS253), 1.9% (58) samples were positive and regarding to the titer distribution, 69.0% (40/58) presented antibody titer 10; 13.80% (8/58) presented final titer value of 14.14213562; 10.34% (6/58) presented titer 20; 1.72% (1/58) had titer 56.56854249; 3.44% (2/58) had titer 40 and 1.72% (1/58) had titer 80.
Conclusion: It was possible to notice that lower titer values were the most common in the tested samples, a fact that could be related to unspecific antibody reaction or even with the fact the swine not being the main host specie for the BVDV. The VN assay was able to detect several variations in the serum antibody titer preliminary suggesting that such diagnostic method is suitable for detecting and quantifying anti-BVDV antibodies in swine serum. This study had financial support provided by grant 2014/13590-3, São Paulo Research Foundation (FAPESP).

Disclosure of Interest: None Declared

Keywords: BVDV, diagnostics, titer values

Viral and Viral Diseases
OTHERS

PO-PF3-271

GEOSPATIAL ANALYSIS OF BOVINE VIRAL DIARRHOEA VIRUS (BVDV) INFECTIONS IN SWINE OF NON-TECHNIFIED HERDS IN THE STATE OF SÃO PAULO, BRAZIL

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Introduction: Recently BVDV infections in swine have been highlighted due this pathogen has some similarities with the classical swine fever virus (CSFV). Information about this type of infection and its epidemiology are an important tool to avoid further problems in official sanitary programs. This study focused on using geospatial analysis tools to describe the BVDV infection's distribution and occurrence in the northeastern region of the São Paulo state, Brazil.
Materials and Methods: All the animals sampled were from a CSF-free zone. A set of 360 serum samples from 56 non-technified herds located in the northeastern region of the state of São Paulo were analyzed by the virus neutralization test to detect the presence of antibodies anti-BVDV in the serum samples. Herds with at least one positive animal had the geographical coordinates subjected to the Kernel intensity estimator tool. To explain the high risk of occurrence areas in the map generated, continuous variables such as: bovine herd size, swine herd size were classified in small, medium or large herd and together with BVDV positivity data, were subjected to a multiple correspondence analysis.
Results: Out of the 56 evaluated herds, 15 had at least one positive animal. The visual assessment of the map generated by the Kernel intensity estimator pointed the existence of three major areas, named area 1, area 2 and area 3 of differentiated risk of occurring BVDV infections. The multiple correspondence analysis identified a strong association ($p < 0.05$) between BVDV-2 infection in swine and the presence of bovine herds with more than 16 animals within the same farm and a moderate association ($p<0.15$) between BVDV-1 infection in swine and the presence of a medium swine herd size (25 to 50 animals). Afterwards, distribution maps of the farms with bovine herds larger than 16 animals and medium swine herd size were generated by the Kernel intensity estimator tool. The new maps of those herds distribution matched the areas 1 and 3 of differentiated risk of BVDV infections occurrence in pigs.
Conclusion: High-risk of infection occurrence areas could be related to the presence of medium size swine herds (25-50 animals) and bovine herds within the farm, highlighting the importance of cattle as the main BVDV infection source for swine and interspecies proximity in the epidemiology of BVDV infections. This study had financial support provided by grant 2014/13590-3, São Paulo Research Foundation (FAPESP).

Disclosure of Interest: None Declared

Keywords: Kernel, multiple correspondence analysis, Pestivirus

Viral and Viral Diseases
OTHERS

PO-PF3-162

Sero-prevalence of Aujeszky disease in a swine production group in east Taiwan: Evaluation of Vaccination Programs

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Introduction: Aujeszky disease is still a major pathogen causing respiratory problem and reproduction failure in Taiwanese swine herds. Though eradication program in breeder farms is in progress, no standard vaccination program recommended for commercial farms. Recently Area Regional Control (ARC) became popular for its benefit of diseases control in an area level. In this study we report a survey on sero-prevalence of Aujeszky disease in a small swine production group in east Taiwan comparing it with their varying vaccination schemes.
Materials and Methods: This serology survey was taken from 5 farms (330-420 sow level) in a small swine production group located at east side of Taiwan. First survey was performed in November 2014 while a second survey was completed in June 2015. At least five serum samples of each age group (sows in varying parities, nursery, grower; pre-finisher and finisher) were collected in each farm. The serum samples were tested for gE antibodies using commercial available Enzyme-Linked Immunosorbent Assay (ELISA) kits (IDEXX PRV gE Ab test kit).
Results: The farms' vaccination programs include pre-farrowing 1 shot with variations of 2 shots in gilts, mass vaccination every 3 or 4 months. Four of the five farms in this group used inactivated vaccines in sows, the other used live vaccine of Bucharest strain. Only one farm vaccinated pigs at 71 days of age. Except farm E which had gE negative sow herd in Q1, all farms are positive to gE in sow and pigs during Q1 and Q2. Sero-prevalence in sow herds were increased in two farms (A & E).
Conclusion: No farm in this study is successful in Aujeszky disease control during the period. Four of the five farms vaccinated with inactive vaccine, indicated that the modified live vaccines are more effective than inactive ones [1, 2]. Only farm A vaccinated with live vaccine, but the strain shown less effective in previous study [1]. Poor gilt selection and flow may also help in the persistence of the virus in the herd. Pig vaccination should not be skipped. Taken together, an appropriate vaccination program with effective vaccine is essential for the successful control of Aujeszky disease.

Disclosure of Interest: None Declared

Keywords: Aujeszky disease, Sero-prevalence, Taiwan

Viral and Viral Diseases
OTHERS

PO-PF3-285

Weaned piglets experimentally infected with Bovine Viral Diarrhea virus: identification of affected organs - preliminary results

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Introduction: Swine can be infected by Bovine Viral Diarrhea virus (BVDV) under natural conditions, for this reason it is needed further information about the action of this virus in swine. The swine may not show apparent lesions or demonstrates a very lightly. The most consistent lesions are transient leukopenia, hyperemia, chronic gastroenteritis and septicemia with bleeding in lymph nodes, epicardium and kidneys. The complete dissemination of the virus in swine occurs in a few days. Thus, the objective of this study was to evaluate the effect and presence of BVDV in organs of weaned piglets experimentally infected.
Materials and Methods: Six seronegative pigs of 21 days had been selected. The zero day (D0) was the day of animal's inoculation and then, placed in the isolators. Randomly divided into three groups in pairs in the insulators. The BVDV Type-1 (Singer strain) was inoculated by the oronasal route with a dose of 1 x 10^5 $TCID_{50}$ in 2 ml. The inoculum was administered through a nasal catheter (0.5 ml per nostril) and 1 mL administered orally, simulating a natural infection. The animals were kept in stainless steel insulators (0,80m x 0,80m x 1,30m), fully enclosed and specially designed for epidemiological studies. The piglets remained in the isolators for 18 days after inoculation, had daily evaluation and, in the end of this period, were euthanized and necropsied to collect samples. Tissue samples collected were: spleen, intestine (ileum portion), mesenteric lymph nodes, mediastinal lymph nodes, inguinal lymph nodes, lung, liver, kidney, tonsil and blood and tested for viral presence. The detection of the presence of virus in organs was performed by nested RT-PCR, thus, samples were submitted to RNA extraction Trizol® (Invitrogen) and obtaining the cDNA by the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to manufacturer's instructions. The identity of the virus was performed using the primer set previously described (WEINSTOCK et al., 2001) for amplifying a 289 bp band.
Results: Positive organs for BVDV presence by nested PCR were: liver, spleen and kidney. The piglets did not show any clinical sign during the experimental period in the insulators and weren't observed gross lesions in the organs at necropsy.
Conclusion: The results demonstrate the main organs for detection of BVDV infection in pigs at molecular diagnosis despite absence of gross lesions. Furthermore, the findings can suggest the virus host cells and the dissemination on the pig body, which may be useful in understanding the pathogenesis of BVDV infection in pigs. This study had financial support provided by grant 2014/13590-3, São Paulo Research Foundation (FAPESP).

Disclosure of Interest: None Declared

Keywords: BVDV, experimental infection, nested RT-PCR

Poster Abstracts

Viral and Viral Diseases
OTHERS

PO-PF3-025

Novel atypical porcine pestivirus (APPV) associated with congenital tremor in newborn piglets

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Introduction: Recently, genomes of a novel genetically highly divergent, so called “atypical porcine pestivirus” (APPV) were discovered in sera of apparently healthy pigs from the USA. Here, we report the identification of APPV genomes in samples of newborn piglets with congenital tremor (CT) as well as in serum of apparently healthy pigs from 2 different Federal states in Germany. CT can be associated with infection, heritability or intoxication.
Materials and Methods: Serum, cerebrospinal fluid and tissue samples from different organs from 6 CT-affected and 2 unaffected piglets as well as serum samples from 23 sows were taken from an acutely affected farm. Additionally, 369 serum samples from clinically unsuspicious sows and fattening pigs (29 farms) were investigated. RNA from liquids and tissues as well as from archived cerebellum samples from 2 piglets with CT necropsied in 2007 was prepared. As no genomes of established pestivirus species were detectable, primers were designed for 2 APPV screening PCRs based on the new available APPV sequence from the USA and the sequences of atypical pestiviruses from bat and rat, targeting conserved regions in the NS3 and the NS4B encoding regions. A large organ spectrum from one piglet with the highest amount of APPV genome and cerebellum from 2 non-shivering piglets as well as from the 2 archived cases were analyzed using a realtime RT-PCR and fluorescent in-situ hybridization (FISH) with a probe targeting a fragment of the NS3 sequence of APPV.
Results: Using the APPV-specific PCRs all of the 6 clinically affected piglets showed APPV genomes in serum, cerebrospinal fluid and pooled brain samples. Archived cerebellum samples from 2 piglets with CT were also positive. Samples of both piglets without tremor and all serum samples of sows from the herd tested negative. Both PCRs identified APPV genomes in 3 sows and 6 finishing pigs from 2 herds from Bavaria and one herd from Lower Saxony without clinical symptoms, respectively.
Quantitative RT-PCR revealed high viral genome loads in lymphoid organs, serum, cerebrospinal fluid, cerebellum and ganglia. FISH detected APPV genome in various organs including glandular epithelial cells, inner granular cell layer of the cerebellum, trigeminal and spinal ganglia as well as in the follicular centers of lymphoid organs. Pathological investigation provided evidence for a mild reduction of myelin accentuated in the spinal cord white mater.
Conclusion: High APPV genome loads detected in the central nervous system of actual and retrospective cases of CT revealed that APPV represents a previously unrecognized pathogen which may contribute to the occurrence of CT in piglets (type AII).

Disclosure of Interest: None Declared

Keywords: atypical pestivirus, congenital tremor, newborn piglets

Viral and Viral Diseases
OTHERS

PO-PF3-245

Analysis of airborne transmission of Bovine Viral Diarrhea Virus among weaned piglets

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Introduction: Bovine Viral Diarrhea Virus (BVDV) has cattle as natural hosts, but it can infect other animals, such as sheep, goat and swine. Once infected, pigs usually do not present clinical signals of infection, which can leads to a silence viral dissemination among animals. Furthermore, the transmission of BVDV between pigs and ruminants requires direct or indirect contact, but virus transmission among pigs remains unknown. Thus, the aim of this work was to evaluate the possibility of BVDV airborne transmission from infected piglets to healthy animals.
Materials and Methods: For analysis of airborne transmission two experiments, one with 18 days and other with 25 days, were conducted using insulators designed for epidemiological studies with weaning piglets. In each experiment, six piglets were divided into three groups, of which the first was the control, the second was compound by the infected animals and the last one was related to the sentinels. The two animals of the second group were inoculated with BVDV type-1, $10^{5,5}$ $TCID^{50}$ viral titer, through nasal catheter and orally. Blood samples were collected by jugular puncturing every four days and nasal swabs samples were collected daily. In the end of experimental period, all piglets were euthanized and necropsied to collect tissue samples (spleen, ileum, mesenteric lymph nodes, mediastinal lymph nodes, inguinal lymph nodes, lung, liver, kidney, tonsil and blood). The seroconversion by the animals was detected through virus neutralization test. The presence of the virus in the nasal swabs and tissue samples was detected by RT-PCR technique using the set of primers designed by Weinstock et al. (2001).
Results: It was not observed the airborne transmission of BVDV among pigs because there was no detection of BVDV in any sample of sentinel groups. Two piglets of the infected group of the first experiment seroconverted on the 16 day after inoculation. However, the excretion of the virus by only one animal was detected on the 17 day after inoculation, which did not persist on the next day. In the second experiment, one piglet of the infected group seroconverted on the 13 day and the other on 22 day after inoculation, but the viral excretion was not detected in nasal swabs samples.
Conclusion: This experimental study demonstrated that the interval between seroconversion and excretion might be only one day. However, the possibility of airborne transmission of BVDV among pigs was not proven within the period evaluated, suggesting that BVDV is not transmitted among piglets in this route, or, it is very limited. This study had financial support provided by grant 2014/13590-3, São Paulo Research Foundation (FAPESP).

Disclosure of Interest: None Declared

Keywords: airborne transmission, BVDV, experimental infection

Viral and Viral Diseases
OTHERS

PO-PF3-159

DESCRIPTION OF AN OUTBREAK OF FOOT & MOUTH DISEASE (FMD) IN A CLOSE CYCLE UNIT IN ISRAEL

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Introduction: This work describes the evolution, the clinical signs and the outcomes of a FMD outbreak in one close-cycle unit in North Israel.
Materials and Methods: A farrow to finish farm, with 350 sows, pregnant or at insemination; 66 after farrowing with 742 piglets; 12 before farrowing; 800 weaners; 820 fatteners; 6 boars. On 19/11/2015 The Veterinary Services were alerted about high mortality in suckling piglets; high fever (>41C) in sows; vesicles and lesions on sows' snouts and feet; reluctance to move. Lesions and mortality were confirmed at inspection on 20/11/2015 and found compatible with FMD. Swine Vesicular Disease (Picornaviridae, Enterovirus), Vesicular Stomatitis (Rhabdoviridae, Vesicolovirus) were never diagnosed in the Country; last FMD outbreak in swine goes back to late '90s. Double blood samples (with, without anti-coagulant); swabs from vesicles of three sows; tongue and claws' tissues samples from an euthanized sow; two dead piglets, were collected and tested for FMD at Kimron Veterinary Institute. Methods used are according to "OIE – Terrestrial Manual 2.1.5; Foot and Mouth Disease" (2012): RT- PCR for all FMD virus types detection; PCR for Type identification; Ag-Elisa for Type identification; MoAb-Elisa for FMD virus identification; NSP antibodies detection (differentiate between vaccination or infection antibodies); CP effect on adult swine kidney cells.
Results: RT-PCR was positive for FMD virus; Cytophatic effect was confirmed; PCR, Ag-Elisa, MoAb-Elisa were positive for FMD Virus Type O. NSP test resulted negative, due to the fact NSP antibodies onset requires at least 10 days, and sampling was done at the beginning of the outbreak (estimated 1st to 6th day, in different subjects). The isolate Type O strain phylogenetically belongs to group O/ME-SA/PanAsia; it was named FMD-Fasuta-15. Typical "tiger heart" lesions were not confirmed in dead piglets.
Conclusion: Time to time FMD outbreaks occur in tne Country; therefore the farm was quarantined and all the swine farm population vaccinated with a trivalent (O, A, Asia 1), double-oil emulsion, inactivated FMD vaccine, 2ml/head; two vaccinations 3-4 weeks apart. The outbreak was considered closed on 25/12/2015, one month following start of vaccination. 66 farrowings were involved in the outbreak, from 13/10 to 16/12/2015, due to piglets weaned older than 30 days of age . Damages were represented by: suckling piglets mortality: 52,81% of total born; 59,43% of live born; still-birth increase to 11,13%; 2 dead sow; 2 euthanized; 9 sent to urgent slaughtering; 5.31% weaners mortality; 0,36% fatteners mortality; one month delay in slaughtering. This is the first description of a FMD outbreak in swine population in Israel.

Disclosure of Interest: None Declared

Keywords: FMD, Israel, mortality

Viral and Viral Diseases
OTHERS

PO-PF3-082

Investigating porcine parvovirus 4 infection using in situ polymerase chain reaction

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Introduction: Porcine parvovirus 4 (PPV4) is a representative of the *Copiparvovirus* genus classified as *Ungulate copiparvovirus 2*. PPV4 was detected recently in the lung lavage samples of diseased pigs co-infected with porcine circovirus type 2 (PCV2). PPV4 is unique in that its genomic sequence is most closely related to bovine parvovirus 2 classified as *Ungulate copiparvovirus 1*. The pathogenic nature of PPV4 remains to be determined while it seems that PPV4 is widespread in pig populations in Central and Eastern Europe. The aim of this study was to detect novel PPV4, using two *in situ* methods that target nucleic acids, like *in situ* hybridization (ISH) and *in situ* polymerase chain reaction (IS-PCR).
Materials and Methods: In order to investigate its role in pathological conditions samples from 46 and 22 dead animals from two farms were examined – necropsied and checked for PPV4 and PCV2 by PCR; tissues embedded in paraffin blocks were submitted to H&E and Brown and Brenn staining, immunohistochemistry (IHC) to detect CD3, CD79α, swine leukocyte antigen class II DQ (SLAIIDQ), lysozyme, PRRSV, swine influenza, *Mycoplasma hyopneumoniae* and *in situ* hybridization to detect PPV4, ss and dsDNA of PCV2. PPV4 positive samples were submitted to IS-PCR including double staining method to detect PPV4 and cell marker.
Results: Out of the 46 lung and lymph node samples tested 6 were positive for the presence of PPV4. PPV4 enhanced necrosis in lymph nodes and was associated with the decreased replication of PCV2 in lymphoid follicles. In lymph nodes PPV4 caused a statistically significant increase in the number of T lymphocytes and was linked to a decreased level of most immune cells including macrophages. PPV4-positive cells were observed in lymph nodes, mostly in the cortex and to some extent in the medulla. Positive cells displayed lymphocyte-like or macrophage-like morphology in haemorrhagic areas where inflammatory cells were mixed with erythrocytes. Only limited number of PPV4-infected foci displayed CD3 antigen in contrast, while in lungs, PPV4-positive cells expressed poorly SLAIIDQ antigen and no CD3 or lysozyme antigens at all. Very discrete microscopic lesions were observed in IS-PCR-positive lungs consisting of lung oedema, congestion and very weak proliferation of alveolar walls.
Conclusion: We found that PPV4 viruses were localized mostly in lymphocytic cells in lungs, lymph nodes and liver. PPV4 could play a role in necrotizing lymphadenitis in the background of PCV2 infection with unclear pathogenesis. Low level of SLAIIDQ and CD3 was expressed by infected cells suggesting that PPV4 may have a specific tropism for immature lymphocytes and/or NK cells.

Disclosure of Interest: None Declared

Keywords: PPV4, in situ PCR

Poster Abstracts

Viral and Viral Diseases
OTHERS

PO-PF3-081

Investigating porcine parvovirus 3 infection using in situ polymerases chain reaction

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Introduction: Parvoviruses are small, non-enveloped icosahedral viruses ubiquitous in different animal species. Porcine parvovirus 3 (PPV3) or according to novel classification *Ungulate tetraparvovirus 2* belongs to the *Tetraparvovirus genus.* PPV3 was found in tissues of healthy and sick pigs. The aim of this study was to detect PPV3, using two *in situ* methods that target nucleic acids, like *in situ* hybridization (ISH) and *in situ* polymerase chain reaction (IS-PCR) and to apply immunohistochemistry in order to detect host immune response or to determine expression of cell markers in infected cells.
Materials and Methods: To investigate its possible involvement in pathological conditions samples from 46 and 22 dead animals from two farms were examined – necropsied and checked for PPV3 including qRT-PCR to determined viral load and PCV2 by PCR; tissue embedded in paraffin block was submitted to H&E and Brown and Brenn staining, immunohistochemistry (IHC) to detect CD3, CD79α, swine leukocyte antigen class II DQ, lysozyme, porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza, *Mycoplasma hyopneumoniae* and ISH to detect PPV3, ssDNA and dsDNA of PCV2. PPV2 positive samples were submitted to *in situ* PCR including double staining method to detect PPV3 and cell markers.
Results: Out of 46 lung and lymph node samples tested 10 were positive for the presence of PPV3. PPV3 level ranged between10^2-10^6 copies per gram of tissue. Reductions of lymphocytic depletion and of histiocytic infiltration were associated with PPV3 infection. Only one lung was positive by IS-PCR and was associated with moderate congestion, mixed inflammatory reaction, weak proliferation of alveolar walls. The alveolar spaces were filled with inflammatory cells were also affected by PRRSV. PPV3 was detected predominantly in bronchus, few areas with positive cells were observed in alveolar spaces in inflamed parts. Cells that expressed positive signal for PPV3 were mostly lymphocyte- and macrophage-like cells. Infected cells poorly expressed SLAIIDQ antigen, and not at all CD3 or lysozyme antigens.
Conclusion: We found that the PPV3 was localized mostly in lymphocytes in lungs, lymph nodes and liver. Neither CD3 antigen nor lysozyme was expressed by these infected cells. In contrast low level of SLAIIDQ was expressed by infected cells suggesting that PPV3 may have a specific tropism for immature B lymphocytes and/or NK lymphocytes but possibly not T lymphocytes. Furthermore we were able to conclude that PPV3 may contribute to the pathogenesis of pneumonia

Disclosure of Interest: None Declared

Keywords: PPV3, in situ PCR

Viral and Viral Diseases
OTHERS

PO-PF3-111

Investigating porcine parvovirus 2 infection using in situ polymerases chain reaction

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Introduction: Porcine parvovirus 2 (PPV2) was detected in swine serum without showing any relation with a disease.The emergence of the virus seemed to be a unique event until other, genetically highly similar parvoviruses were identified in China while later in 2012 the presence of the virus was also described in Europe. Phylodynamic analysis indicated that PPV2 must have been around in Europe since 1920 in domestic and sylvatic hosts. It seems that PPV2 is widely distributed in pig populations and it is suspected to be involved in respiratory conditions of pigs, based on the frequent detection of the virus in lung samples
Materials and Methods: In order to investigate its possible implication in pathological conditions samples from 46 and 22 dead animals from two farms were examined – necropsied and checked for PPV2 and PCV2 by PCR; tissue embedded in paraffin block was submitted to H&E and Brown and Brenn staining, immunohistochemistry (IHC) to detect CD3, CD79α, swine leukocyte antigen class II DQ (SLAIIDQ), lysozyme, porcine reproductive and respiratory syndrome virus, swine influenza, *Mycoplasma hyopneumoniae* and *in situ* hybridization to detect ssDNA and dsDNA of PCV2. PPV2 positive samples were submitted to *in situ* PCR (IS-PCR) including double staining method to detect PPV2 and cell marker
Results: We found that PPV2 increased intensity of bleedings in lymph nodes and was associated with the increased amount of PCV2 in lymph follicles and decreased replication of PCV2 in lymph follicles, cortex and around blood vessels, appeared to cause higher reduction of alveolar spaces as well as more inflamed cells in alveolar spaces. PPV2 was detected in lungs, lymph nodes, liver and spleen in 4 out of 22 animals of the second farm. Using IS-PCR moderate to severe infection of PPV2 was detected in three lungs. Extensive areas were infected and positive signals were mostly found in lymphocyte- and macrophage-like cells in alveolar space. PPV2-positive lungs were associated with strong congestion, lymphocytic infiltration, reduction of alveolar spaces, and necrosis of ciliary epithelial. PPV2 infected cells did not express any of the studied markers or mainly but very poorly the SLAIIDQ antigen.
Conclusion: We found that the PPV2 was localized mostly in lymphocytes in lungs, lymph nodes and liver. Neither CD3 antigen nor lysozyme was expressed by these infected cells. In contrast low level of SLAIIDQ was expressed by infected cells suggesting that PPV2 may have a specific tropism for immature B lymphocytes and/or NK cells but possibly not T lymphocytes. We were able to conclude that PPV2 may contribute to the pathogenesis of pneumonia.

Disclosure of Interest: None Declared

Keywords: None

Viral and Viral Diseases
OTHERS

PO-PF3-112

Epidemiological investigation of cases of senecavirus A in United States swine breeding herds

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Introduction: The objective of this report was to describe the findings from epidemiological investigations to characterize clinical features and identify likely routes by which senecavirus A (SVA) was introduced into sow herds in the Midwest USA.
Materials and Methods: Sow farms investigated were enrolled from cases submitted to the Iowa State University Veterinary Diagnostic Lab (ISU VDL) where SVA was confirmed by PCR. A standard Epidemiologic Investigation Form was used to collect information in a manner suitable for aggregate analysis. The form was adapted from one developed for the PRRS Outbreak Investigation Program, funded by the Iowa Pork Producers Association (IPPA). Information was collected about the herd, premises, outbreak and a comprehensive list of risk events, such as swine movements and entry of people, and the carrying agents associated with them. Risk events were assigned a risk level of high, medium, or low based on the frequency of the event, likelihood that carrying agent(s) associated with the event were contaminated or infected with SVA and the likelihood that SVA was transmitted from carrying agent(s) to pigs in the herd.
Results: Investigations were conducted on a farrow-to-finish herd in Iowa (1) and farrow-to-wean herds in Illinois (1), Iowa (1), Minnesota (1), and Nebraska (2). All 6 farms reported increases in pre-weaning mortality (PWM) that resolved within 3 weeks. All 6 reported that sows were anorexic during the acute outbreak. A mild to moderate piglet scour was reported in (3/6) cases. Vesicular lesions on the nasal and coronary bands of sows were reported in 4/6 cases. The prevalence of vesicular lesions ranged from 10 to 70% of sows in the herd. In one case, 90% of the sows were severely lame. The frequency of risk events varied, from 132 to 757 in the 4 week period before the onset of clinical signs, depending on the size of the farm and farrowing system (continuous versus batch) used. Events rated as high risk for SVA introduction by the investigation team were: on-farm employees (4/6 farms), dead disposal (4/6), cull sow removal (3/6) and replacement gilt entry (2/6).
Conclusion: The clinical presentation was variable. Outbreaks occurred on farms of all sizes in swine dense and non-dense areas. Of the farms investigated, 3 had relatively low levels of biosecurity, including the absence of showering procedures. One had relatively good biosecurity, but a high frequency of risky events due to the large size of the farm. For the other 2 farms, the biosecurity was average but operational connections with other positive farms or major deviations from protocols were identified as likely responsible for the outbreaks.

Disclosure of Interest: None Declared

Keywords: Epidemiology, outbreak, Senecavirus

Viral and Viral Diseases
OTHERS

PO-PF3-166

MULTIPLEX PCR FOR SIMULTANOUS DIFFERENTIATION BETWEEN VACCINE AND WILD STRAIN OF AUJESZKY'S DISEASE VIRUS IN KOREA

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Introduction: Aujeszky's disease virus (ADV) causes the Aujeszky's disease (AD), also known as pseudorabies, and infects the central nervous system and several organs in various animals except humans and the tailless apes. Specially, pig is the natural host and the reservoir host with latently infected state. The AD is endemic in many countries, but several countries such as USA, Canada, and many European countries successfully eliminate this disease. In June 1987, first AD outbreak occurred in pig farm in Yangsan district of Gyoungnam province in Korea. Total 1503 cases were detected from 1987 to 2009, and no outbreak occurred since 2010 in Korea. However, the sero-positive rate against ADV was 3.55% (8/225) in wild boar sera collected between March and August 2013. Yangsan (YS) strain was isolated in 1987 and YS400 (ΔTK+IL2ΔgE+βgal) vaccine strain was developed using YS strain for live recombinant vaccine in Korea. In this study, differential multiplex PCR assay containing three primer sets was assessed in order to develop diagnostic method for rapid detection and differentiation of antigen between vaccine and wild strain in the surveillance and control program against ADV.
Materials and Methods: Three primer sets were designed in UL24 and IL2 (360 bp, for vaccine strain), gD (810 bp, for both ADV) and gE (549 bp, for wild strain) genes of YS400 vaccine and YS (Yangsan) wild strain. Differential multiplex PCR assay was developed using mixture of three primer sets in one tube for simultanous differentiation between ADV vaccine and wild strain. In order to test differential ability, detection limit, and specificity of the differential multiplex PCR assay, various ADV wild and vaccine strains, and other swine viral pathogens were used in the study.
Results: The 549 bp and 810 bp specific two bands were shown in various ADV wild strains, whereas the 360 bp and 810 bp specific two bands were detected in the YS400 (ΔTK+IL2ΔgE+βgal) vaccine strain. The 810 bp primer set is designed for common detection of ADV wild and vaccine strains. Therefore other gE-deleted vaccine Bartha strain (ΔgIΔgE) was also detected by the 810 bp primer set. The specificity of the differential multiplex PCR assay was assessed using other viral pathogens such as classical swine fever virus (CSFV), porcine circovirus (PCV) and so on. There was no positive band in the other viral pathogens except ADV.
Conclusion: The differential multiplex PCR assay containing three primer sets for vaccine, wild and both strain was successful in detecting DNA of ADV wild and gE deleted vaccine strain. Therefore, the multiplex PCR assay would be a diagnostic method for rapid and accurate detection and differentiation between ADV vaccine and wild strain.

Disclosure of Interest: None Declared

Keywords: Aujeszky's disease virus, differential multiplex PCR, pseudorabies

Poster Abstracts

Viral and Viral Diseases
OTHERS

PO-PF3-165

Development of indirect ELISA for the detection of antibodies against Japanese encephalitis virus in swine

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Introduction: Japanese encephalitis virus (JEV) transmitted by mosquitoes is caused reproductive failure in pregnant sow and the encephalitis in human. Therefore, it has been known as zoonosis. There are several methods to detect antibodies against JEV. These methods include hemagglutination inhibition (HI), viral neutralization test (VN), plaque reduction neutralization test (PRNT) and enzyme-linked immunosorbent assay (ELISA). HI test is considered the standard method to detect antibody against JEV. However, the complicated test procedure may limit the use of HI test. The VN and PRNT test are more reliable method. However, both of the tests take one week to get the results. ELISA test is the most rapid and easiest method. Many ELISA products related to JEV have been developed in human field but ELISA products for animal are rare. In this study, we developed indirect ELISA (I-ELISA) for swine and estimated the efficacy by compared with HI, VN and PRNT.
Materials and Methods: A total of 175 swine serum samples were collected from slaughterhouse and Yeonggwang province. KV1899 strain of the JEV which was isolated from swine blood in 1999 was used for HI test, $PRNT_{90}$ and I-ELISA. K87 strain which was isolated from mosquito was used for VN test in this study. HI test was carried out in 96 well microplates, using a little modified method of Clarke and Casals. The HI titer of 1: 20 or higher was considered positive. $PRNT_{90}$ titers ≥1:10 were considered positive. The antigen and swine serum was diluted from 4 to 1 μg/mL and 1:2 to 1: 256 to detect optimal concentration of antigen and serum dilution for I-ELISA, respectively. VN test was conducted by micro neutralization test technique. An antibody titer ≥ 1:2 was considered positive.
Results: The optimal antigen concentration and serum dilution were found to be 2μg/mL and 1:100, respectively. The absorbance >0.25 was considered positive. The absorbance results obtained from 175 swine serum samples were compared with the results of VN, HI and $PRNT_{90}$. The significant correlation was shown between VN (r=0.95), HI (r=0.93) and $PRNT_{90}$ (r=0.95). The sensitivity of I-ELISA was 95.0% with VN test, 91.8% with HI test and 94.7% with $PRNT_{90}$, respectively. The specificity was 94.7%, 92.2% and 94.7% with the VN, HI and $PRNT_{90}$, respectively.
Conclusion: ELISA test is the easiest method and is suitable for large number of samples compared with VN, HI and PRNT. In addition, it is easy to test many samples without special equipments at one time. According to the results, I- ELISA showed a high correlation with other methods, which suggests that I-ELISA can be used for the detection of antibodies against the JEV in swine.

Disclosure of Interest: None Declared

Keywords: enzyme-linked immunosorbent assay, Japanese encephalitis virus, swine serum

Viral and Viral Diseases
OTHERS

PO-PF3-087

Concentration, size distribution, and infectivity of airborne particles carrying PRRS, influenza A, and PED viruses emitted by acute infected animals

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Introduction: When pathogens become airborne, they travel associated with particles of different size and composition. Particle size determines the distance across which pathogens can be transported, as well as the site of deposition and the survivability of the pathogen. Despite the importance of this information, the size distribution of particles bearing viruses emitted by infectious animals remains unknown. In this study, we characterized the concentration and size distribution of inhalable particles that transport influenza A virus (IAV), porcine reproductive and respiratory syndrome virus (PRRSV), and porcine epidemic diahrrea virus (PEDV) generated by acutely infected pigs, and assessed virus viability for each particle size range.
Materials and Methods: Aerosols from experimentally infected pigs were sampled for 24 days using and Andersen cascade impactor able to separate particles by size (ranging from 0.4 to 10 micrometers (μm) in diameter). An optical particle counter was used to analyze total airborne particles during the sampling periods. Oral fluids, clinical signs, coughing and lethargy scores were collected daily. Air samples collected for the first 9, 20 and the last 3 days of the stuyd were analyzed for IAV, PRRSV, and PEDV respectively using quantitative RT-PCR and quanitified as a geometric mean copies/m^3 within each size range. Virus viability was tested on specific cell cultures.
Results: IAV was detected in all particle size ranges in quantities ranging from $5.5x10^2$ (in particles ranging from 1.1 to 2.1 μm) to $4.3x10^5$ RNA copies/m^3 in the largest particles (9.0-10 μm). PRRSV was detected in all size ranges except particles between 0.7 and 2.1 μm in quantities ranging from $6x10^2$ (0.4-0.7μm) to $3.5x10^8$ RNA copies/m^3 (9.0-10μm). PEDV, an enteric virus, was detected in all particle sizes and in higher quantities than IAV and PRRSV ($p<0.0001$) ranging from $1.3x10^6$ (0.4-0.7 μm) to $3.5x10^8$ RNA copies/m^3 (9.0-10.0μm). Infectious status was demostrated for the 3 viruses, as a positive bioassay with the air samples for PEDV, and in the case of IAV and PRRSV, both viruses were isolated from particles larger than 2.1 μm.
Conclusion: We investigated the particle concentration, size distribution, and infectivity of 3 animal viruses with different pathogenensis and transmission routes. IAV, PRRSV and PEDV emitted by infected pigs were found associated with a wide range of particle sizes that can deposit throughout the respiratory tract and later be swallowed. However, we demostrated that viability of airborne viruses was particle size dependent with IAV and PRRSV isolated only from particles > 2.1μm. Our results support the relevance of the aerosol route in the transmission of IA, PRRS and PED viruses.

Disclosure of Interest: None Declared

Keywords: Particle size, Swine aerosols, Virus viability

Viral and Viral Diseases
OTHERS

PO-PF3-033

The Eukaryotic virome of neonatal piglets suffering from new neonatal porcine diarrhoea syndrome

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Introduction:
A new neonatal porcine diarrhoea syndrome (NNPDS) unresponsive to traditional prophylaxis and treatment has been reported from a number of European countries during the last years. However, the aetiology of this condition remains uncertain. This study aimed at describing the intestinal virome of piglets with neonatal porcine diarrhoea of uncertain aetiology by the use of viral metagenomics in an effort to discern a possible viral involvement.
Materials and Methods:
Samples from the distal jejunum of 50 diarrhoeic and 19 healthy piglets from 10 affected herds were analysed. The viral fraction of the samples was isolated and subjected to sequence independent amplification before sequencing. Samples from diarrhoeic piglets from the same herds were pooled whereas samples from healthy piglets were analysed individually. Samples were sequenced using the Ion Torrent technology on the Ion Proton platform. The resulting sequence data was subjected to taxonomic classification using KRAKEN with a customized database enriched with viral sequences.
Results:
Sequencing data averaged 220Mbases at a mean read length of 185 bases. Mammalian viruses were detected in five out of ten pooled diarrhoeic samples and 14 out of 19 individual samples from the healthy controls. In the healthy specimens, eight different mammalian virus families were detected (Adenoviridae, Anelloviridae, Astroviridae, Caliciviridae, Circoviridae, Parvoviridae, Picornaviridae, Reoviridae) compared to five in the pooled diarrhoeic samples (Adenoviridae, Anelloviridae, Picornaviridae, Reoviridae).
Conclusion:
The data did not support an association between the diarrhoea and previously known mammalian viruses. There were however several eukaryotic viruses present in the samples of both healthy and sick animals. Since most samples were collected within 24-48 hours of birth this also indicates immediate post-partum infection or possibly transplacental infection. The findings provide important information on the developing virome of neonates as well as a starting point for further investigations of neonatal porcine diarrhoea of uncertain aetiology.

Disclosure of Interest: None Declared

Keywords: Metagenomics, Porcine, Virome

Viral and Viral Diseases
PCV2

PO-PT2-218

CIRCOVIRUS (PCV2-2) rtPCR DETECTION FROM HEART TISSUE IN MUMMIES OF DIFFERENT PARITIES

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Introduction: Introduction: PCV2 infection has been involved in several reproductive pathology, virus infect different fetal tissues from early gestation stages with different implications such as re-absorptions, mummies and abortions affecting herd reproductive performance. Heart is consider the diagnostic tissue target to detect it. Different reports consider PCV2 as a common reproductive infection including boar semen shedding, even vaccination in herd is common routine diagnostics in mummies or abortions is not common (1,3).**Objective:** Detect PCV2 infection in heart tissue from different parity mummies
Materials and Methods: Material and Methods: 303 mummies selected from nine farms located in four country regions were separated by it parity origin, they were frozen at -20ºC until laboratory process. Mummies were measure from tail to head to obtain approximate age and heart was dissected aseptically. Cardiac tissue were used to perform a PCV2-rtPCR
Results: Results. From the nine farms six were PCV2 positive in some parity, first parity was the common detection, detection expressed as particles/ml the average for parity 1 was; 8.22 x 10^9 parity 2; 7.78 x 10^7 parity 3; 8.02 x 10^7 parity 4; 1.7 x 10^8 and parity ≥ 5; negative. Positive farms trend to show mummies in first parities while negative ones are distributed in all parities. Positive farms show younger mummies (50 to 70 days) than negative ones (80 to 100 days). Fetal immune response began at 70 gestation days, negative farms show mummies in older ages.
Conclusion: Discusion. Although all positive farms vaccinate reproductive herd at least once, we detect PCV2 in first parities mainly, ages are younger than other infections because fetus can response after 70 days age to a PCV2 challenge (2). Some reports indicate the vertical PCV2 fetal infection where piglets born infected. In other situations PRRS and PCV2 could affect at the same time with confusion during the diagnostic process (3) **Conclusion.** PCV2 continue affecting the herd performance, it´s subclinical form and the infection by the replacement gilts close the natural infectious cycle. It´s important to discard PCV2 when the herd prolificity is affected.
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Disclosure of Interest: None Declared

Keywords: mummies, parity, rtPCR

Poster Abstracts

Viral and Viral Diseases
PCV2

PO-PT2-185

Identification and Functional Analysis of the Novel ORF4 Protein Encoded by Porcine Circovirus Type 2

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Introduction: Porcine circovirus type 2 (PCV2) is the primary causative agent of porcine circovirus-associated diseases in pigs. Previous studies have described three viral proteins named Cap, Rep, and ORF3 . In this study, a novel viral gene within the PCV2 genome (termed ORF4) was determined at the transcriptional and translational level and functionally analyzed in vitro. Northern blot analysis indicated that the ORF4 gene is about 180 bp and overlaps ORF3 in the same direction. Expression of ORF4 was abviously detected by specific mAbs.Site-directed mutagenesis confirmed that the viral ORF4 protein is not essential for virus replication in PK-15 cells infected with an ORF4-deficient PCV2 (PCV2$^{\triangle}$). Moreover,PCV2$^{\triangle}$ triggered higher activity levels of caspase-3 and-8 than wild-type PCV2 (wPCV2) in PK-15 cells , indicating ORF4 protein might involve in antiapoptosis pathway. These results provide novel information on elucidating the molecular mechanisms of PCV2 pathogenicity.
Materials and Methods: The PCV2 strain HZ0201(AY188355) ,ORF4-deficient PCV2 (PCV2$^{\triangle}$) and MAbs against PCV2 Rep ,Cap and ORF4 protein were maintained in our laboratory. ORF-specific DNA/RNA probes were generated through Related kits(Roche). Northern blotting assay identified the ORF4 transcript. Indirect immunofluorescence assay (IFA) and Immunoblot analysis ORF4 protein expression. The titers of viruses were determined by calculating TCID50. Proteolytic caspase activities were detected using ApoAlert caspase-3 and caspase-8 colorimetric assay kits (Clontech) and a caspase-9 colorimetric assay kit (Millipore).
Results: Northern blotting assay detected three RNA bands in total cellular RNA of wPCV2-infected PK-15 cells,among them, the 180bp band was identical to the size of the putative ORF4 transcript.Moreover,the ORF4 was detected in the same orientation as ORF3. Expression of the PCV2 ORF4 protein in wPCV2-infected PK-15 cells was observed by IFA and confocal microscopy as early as 24 hpi and peaked at 60 hpi,however no ORF4 protein signal was visualized by Western blotting. The titer of PCV2$^{\triangle}$was similar to that of wPCV2 over the course of infection indicating that the viral protein ORF4 was not essential for PCV2 replication in PK-15 cells. Proteolytic caspase activities assay showed the proteolytic activities of caspase-3, caspase-8, and caspase-9 were induced by both PCV2$^{\triangle}$ and wPCV2. At 24 and 72 hpi, the activities of caspase-3 and -8 induced by PCV2$^{\triangle}$ were higher than those of wPCV2.
Conclusion: ORF4, a novel identified protein,was not essential for PCV2 replication,however,might play an critical role in suppressing apoptosis during PCV2 infection.

Disclosure of Interest: None Declared

Keywords: apoptosis, ORF4, PCV2

Viral and Viral Diseases
PCV2

PO-PT2-204

Characterization of IgG levels against porcine circovirus type 2 and virus load after vaccination of pigs under field conditions: A longitudinal study

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Introduction: PCV2 is associated with different diseases of swine known as Porcine Circovirus Associated Diseases (PCVAD). Piglets are vaccinated against PCV2 to reduce the impact of PCVAD. However, the difference on antibody response and virus load of PCV2 between animals vaccinated under different vaccination protocols are not completely understood. The objective of this study was to compare the humoral immune response and virus load in a cohort of pigs after weaning using two different vaccination protocols.
Materials and Methods: Two breeding farms (FA and FB) were selected for this study. Both farms use a recombinant PCV2 vaccine expressed in a baculovirus. FA vaccinates gilts (at arrival), all sows (every 6 months) and piglets (at weaning). FB vaccinates gilts at arrival and piglets at 3 and 5 weeks of age. In each farm, 40 pigs were selected at weaning to estimate the association between antibody response after vaccination and virus load over time. 10 out of 40 pigs in each farm were maintained unvaccinated and used as control groups. Serum samples were collected from all pigs at weaning and every 4 weeks until 23 weeks of age. IgG titers (INGEZIM Circovirus IgG kit, Ingenasa-Spain) and virus load (qPCR) were measured in all samples. Samples were considered positive when the PCR cycle threshold (Ct) value was lower than 35. Mean IgG and PCV2 copies/ml were compared within and between farms and considered statistically different between groups if the Kruskal Wallis test p value was lower than 0.05. The linear association between ELISA titters and the viral load was estimated at each sampling event and considered statistically significant at the same confidence level (0,05).
Results: In average vaccinated piglets at weaning from FA had higher IgG titers than piglets from FB ($p<0.05$). However, vaccinated piglets from farm B had higher IgG titers at 7, 11, 15, 19 and 23 weeks of age ($p<0.05$). There was no statistical difference in the IgG titers between vaccinated and non-vaccinated pigs in farm A ($p>0.05$). In contrast, vaccinated pigs in farm B had higher IgG titers than non vaccinated pigs after 7 weeks of age ($p<0.05$). Moreover, the highest number of PCV2 copies per ml among all samples was $1.07x10^4$ and therefore positive pigs were not considered viremic. Although, the IgG titers in farm B were higher than the IgG titers in farm A the virus load was not different ($p>0.05$) between vaccinated pigs in farm A and vaccinated pigs in farm B.
Conclusion: In conclusion, two-dose vaccination against PCV2 in piglets can provide higher IgG levels in pigs after weaning. However, lower titers are not necessarily associated with higher virus loads of PCV2.

Disclosure of Interest: None Declared

Keywords: humoral immunity, VACCINATION, virus load

Viral and Viral Diseases
PCV2

PO-PT2-253

Porcine circovirus type 2 quantification and humoral immune profile in sows and piglets under different vaccination protocols

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Introduction: PCV2 is endemic in swine and is associated with different diseases in pigs. Sow vaccination provides maternally-derived antibodies (MDA) to piglets and may reduce the incidence of diseases associated to PCV2. In this study we characterized the virus load and humoral immune profile in sows and piglets in two commercial sow farms using two different vaccination protocols.
Materials and Methods: Two breeding farms (FA and FB) were used for this study. Both farms use a PCV2 recombinant vaccine expressed in a baculovirus. FA vaccinates gilts at arrival and all sows twice a year. FB vaccinates only replacement animals at arrival. In each farm 45 piglets were selected from 9 sows at farrowing. Serum samples were collected from sows 2 weeks before farrowing and at farrowing. Serum samples were also collected from piglets at birth and every week until weaning. Samples were tested for IgG and IgM (INGEZIM ELISA, Ingenasa-Spain) and PCV2 was quantified by qPCR. Mean IgG, IgM titers, and PCV2 copies/ml were compared between farms and considered statistically different at 0.05 using the Kruskal Wallis test. The linear association between ELISA titters and virus load was estimated and considered statistically significant at the same confidence level ($p<0.05$).
Results: 186 and 187 serum samples were collected from FA and FB respectively. All sows had IgG or IgM antibodies against PCV2 and all piglets tested negative for antibodies against PCV2 at birth. Although two weeks before farrowing the IgM levels in sows between farms was not statistically different ($p=0.83$), sows in FB had higher antibody levels at all other sampling events ($p<0.05$). Additionally, piglets in FA did not test positive for IgM at any point during the study period while piglets in FB were positive for IgM at weeks 1, 2 and 3. Although piglets in both farms had IgG titers at weeks 1, 2, and 3, titers were always higher ($p<0.05$) in piglets from FB. Moreover, the highest number of PCV2 copies found in sow and piglets samples was $1.72x10^3$ and $1.74x10^4$ copies/ml respectively. The number of PCV2 copies per ml was higher in sows from FA than from FB ($p<0.05$) although in average it was lower than 300 copies per ml in both farms. The number of PCV2 copies per ml was higher ($p<0.05$) in piglets from FA compared to piglets from FB at weeks 1, 2, and 3 although there was no association between any antibody levels and virus loads compared ($p>0.05$).
Conclusion: Our results indicate that vaccinating sows every six months can strength the humoral immune status in sows at farrowing and piglets before weaning. However, low viral loads were found in both farms regardless of the vaccination protocol used.

Disclosure of Interest: None Declared

Keywords: humoral response, sow vaccination, virus load

Viral and Viral Diseases
PCV2

PO-PT2-271

Effects of PCV2 vaccines on pig performance

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Introduction: In many cases vaccination against PCV2 and Mycoplasma hyopneumoniae is done around weaning when piglets have to cope with many stressors. The pig's performance around weaning is critical for the later performance and the degree of variability after weaning has a substantial impact on the variability at the end of finishing. The objective of this trial was to determine whether the negative impact of a vaccine on the weight gain shortly after vaccination has an impact on the weight gain until slaughter.
Materials and Methods: The trial was conducted in a French farrow to finish 1200 sows PRRSv negative farm. From weaning to slaughter the mortality rate in this farm is usually about 4% and the feed conversion rate is around 2.55. In total, 1158 pigs of 2 following farrowing batches were included in the study. One day before weaning, the piglets were weighed individually, randomly allocated to either Group 1 or Group 2 and marked individually with an ear tag. Piglets in Group 1 were vaccinated with Ingelvac MycoFLEX (1 ml) and Porcilis PCV (2 ml) whereas piglets in Group 2 received Ingelvac MycoFLEX and Ingelvac CircoFLEX (1 ml of each vaccine, freshly mixed). In addition, 10 non-vaccinated sentinel piglets per batch were included to assess the PCV2 infection status. The piglets were weighed again individually 14 days after vaccination. Average Daily Gain at slaughter was calculated considering the number of days to slaughter. Data between the groups was compared using a t-test except losses using a χ^2 test.
Results: At inclusion mean body weights, sex ratio, parity as well as age were similar between the two treatment groups. The PCV2 PCRs as well as the Elisa tests on the 10 sentinels indicates that the pigs did not get infected with PCV2 during the study.In total, 28 pigs died during the study, 14 in each treatment group. 14 days after vaccination, the ADG was significantly greater ($p<0.0001$) in Group 1 (199.7 g/day) than in Group 2 (216.3 g/day). At slaughter, ADG was 698.3 g/day in Group 1 and 706.4 g/day in Group 2. The significant difference observed 14 days after vaccination was sustained until slaughter ($p=0.045$).
Conclusion: The outcome of this study indicates that the differences in local and systemic reactions between commercial PCV2 vaccines that can be observed shortly after vaccination have an impact on weight gain until slaughter. Since there was no evidence of a PCV2 circulation, this study demonstrates that the safety and not only the efficacy of a vaccine should carefully be considered when choosing a PCV2 vaccine as it might impact the overall pig performance.

Disclosure of Interest: None Declared

Keywords: PCV2 vaccine, performance

Poster Abstracts

Viral and Viral Diseases
PCV2

PO-PT2-183

Success of Porcine Circovirus eradication in a specific pathogen free miniature pig herd by repeated vaccination

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Introduction: Porcine Circovirus Type 2 (PCV2) is the causative pathogen for porcine circovirus-associated disease (PCVAD). To research the pathogenicity and etiology of PCVAD, a PCV2 free herd is necessary. Furthermore, PCV2 must be eradicated for an animal to be a donor for xenotransplantation White Yucatan miniature pigs are commonly bred for xenotransplantation. In 2009, the International xenotransplantation association (IXA) designated that 76 viruses need to be eradicated from all source animals. PCV2 is not a dangerous pathogen to humans, but it is undesirable because it indicates a breakdown in biosecurity and/or may compromise the health of the pigs. Unfortunately, as PCV2 transferrable through the placenta, it is very difficult to eradicate.
Materials and Methods: Optipharm imported miniature pigs from the Sinclair Research Center (MO, USA) in 2007. All miniature pigs were maintained in a barrier facility with a HEPA filtered AHU (air handling unit). The imported miniature pigs were antigen and antibody positive for PCV2. Beginning in 2008, a PCV2 eradication program was designed. To eradicate PCV2 in sows, PCV2 commercial vaccines were administered a total of four times to 83 miniature pigs. The Synbiotics PCV2 antibody ELISA kits were used for PCV2 antibody analysis.
Results: The last PCV2 antigen was detected in June 24, 2009. After that, PCV2 antigen was not detected in any sows or piglets. However, antibody testing was still positive. When maternal antibodies were not present, no seroconversion was observed. It took an average of 140 days for maternal antibodies to disappear. Since 2009, PCV2 vaccination has ended. Although the PCV2 virus was eradicated, vaccinated sows are producing piglets; therefore, maternal antibodies are still detected. Since 2012, all sows and piglets were serologically negative for PCV2. Finally, all miniature pigs became a PCV2 naïve herd.
Conclusion: Repeated commercial vaccine inoculation made it possible to eradicate PCV2 in an Specific Pathogen Free (SPF) facility. The SPF facilities are maintained with HEPA filtered air, and the density of pigs is very low compared to general commercial pig farms. However, in this experiment, we showed that PCV2 can be eradicated after repeated commercial vaccine administration. The successful eradication of PCV2 makes it possible to evaluate the PCV2 vaccine efficacy without the indirect effects of humoral immunity induced by PCV2. Additionally, by monitoring for PCV2, its recurrence can be an indicator of a breakdown in the SPF biosecurity system.

Disclosure of Interest: None Declared

Keywords: Eradication, PCV2, vaccine

Viral and Viral Diseases
PCV2

PO-PT2-243

Comparison of porcine circovirus type 2 (PCV2) antibody and genome detection on serum and oral fluid samples in animals vaccinated at different ages

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Introduction: Porcine circovirus type 2 (PCV2) antibody and genome detection in serum are commonly used methods for infection assessment. During last years, oral fluids (OF) have been also used to detect antibodies and viruses. The objective of this study was to compare antibody detection and real time quantitative PCR (qPCR) techniques applied on serum and OF samples in pigs vaccinated against PCV2 at different ages.
Materials and Methods: At 2 weeks of age (woa), 452 piglets were selected and distributed (at 3 woa) in 4 balanced treatment groups. Pigs from groups 1, 2 and 3 were vaccinated with a single dose of CIRCOVAC® 0.5 mL IM at 3, 6 and 10 woa, respectively, and pigs from group 4 were kept unvaccinated. Blood samples were taken from 80 animals (2-4 piglets were bled per pen in nursery and 2 piglets per pen in fattening) at 6, 10, 14, 18 and 25 woa. OF samples were collected from these pens at the same sampling points. Presence of PCV2 antibodies were determined in serum (Ingezim Circo IgG 11.PCV.K1®, Ingenasa) and OF samples (protocol used at Labocea, Ploufragan-personal communication). Moreover, PCV2 load was also detected using qPCR (LSI VetMAX™ PCV2-Quantification, Life Technologies) for both serum and OF.
Results: Pigs vaccinated at 3 and 6 woa showed a significant ($p<0.05$) increase in ELISA S/P values at 10 woa. In groups vaccinated at 10 woa and non-vaccinated the antibody response was detected at 14 and 18 woa, respectively. In OF, the same serological pattern was observed in all groups, with the exception of the pigs vaccinated at 6 woa, which did not seroconverted by 10 weeks of age. The number of viremic animals was lower in groups vaccinated at 3 and 6 woa compared to the control group ($p<0.05$) at 14 and 18 woa. On average, pigs vaccinated at 3 and 6 woa experienced a lower viral load in serum than those left unvaccinated or vaccinated at 10 woa ($p<0.05$). Viral load values obtained from OF were higher than those found in serum, mainly at 6 woa ($p<0.05$), and no significant differences between groups were observed.
Conclusion: In this study, antibody detection techniques in both samples offered similar results, which suggest their usefulness to study the PCV2 antibody dynamics. In contrast, despite of the high qPCR positivity in OF, PCV2 vaccination at 3 and 6 weeks of age achieved to reduce PCV2 infection in serum samples. OF sampling is giving the picture of collective samples. However, since PCV2 is likely to be present in the pig environment, detection in OF should remain only a raw indicative method.
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Disclosure of Interest: None Declared

Keywords: Diagnosis, Porcine circovirus type 2, Vaccine

Viral and Viral Diseases
PCV2

PO-PT2-287

PORCILIS® PCV M HYO SAFETY COMPARED AGAINST OTHER COMMERCIAL PCV AND MYCOPLASMA HYOPNEUMONIAE COMBINATION VACCINE

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Introduction: Piglets are quite frequently vaccinated against both *M.hyopneumoniae* (Mhyo) and PCV2. Porcilis® PCV M Hyo is a newly developed vaccine that provides double protection against PCV2 and Mhyo in a ready-to-use single administration. The objective of this trial was to compare safety of Porcilis® PCV M Hyo to another commercially available combination vaccine in two commercial farms by evaluating fever and effect on growth during the nursery period.
Materials and Methods: The study was conducted in two farms in Northern Spain (Zaragoza) in March 2015. Both farms have a two phase production system with 500 sows each. Both farms used a PCV2 and Mhyo vaccine at 3 weeks of age. For the study, piglets were randomized at weaning in two equal groups and were divided in Group A - vaccinated with Porcilis® PCV M Hyo and in group B – other PCV2 and Mhyo combination vaccine. Animals were monitored for local and systemic effects at the time of vaccination and for the next 48 hours. Within the weaned group, 50 animals between the two farms were checked for individual body temperature, at the time of weaning, at 24 and 48 hours post vaccination. Piglets were weighed individually in farm 2, (100 piglets), at the time of vaccination (weight 1), 3 weeks later (weight 2) and 6 weeks post vaccination, when they transfer to the fattening unit (weight 3). All data were statistically analyzed using ANOVA, Levene test.
Results: No local post-vaccination reactions were recorded in either group. Rectal temperature in farm 1 was: Group B:Tª 0 - 39.68, Tª 24h - 38.71, Tª 48h - 38.69 and group A :Tª 0 - 39.53, Tª 24h - 38.77 , Tª 48h - 38.83 ($p>0,05$). In farm 2, group B: Tª 0 - 39.4, Tª 24h - 39.8, Tª 48h - 39.15 and group A Tª 0 - 38.9, Tª 24h - 39.2, Tª 48h - 38.95 ($p<0,05$ in Tª0 and Tª 24h, $p>0,05$ in Tª 48h). Weights were higher at all weighing points, but difference was only significant at weight 2: weight 1: 5.92kg group B vs 6.08 kg group A ($p = 0, 446$); weight 2: 8.83kg group B vs 10.25kg group A ($p=0,003$); weight 3: 16,30kg group B vs 18,25kg group A ($p=0,756$).
Conclusion: The lack of difference in rectal temperature and performance during the post-vaccination phase support that Porcilis® PCV M Hyo is safe when used in commercial farms. Porcilis PCV M Hyo pigs also had numerically higher weight gain at the end of the nursery phase than the control pigs.

Disclosure of Interest: None Declared

Keywords: Circovirus, Mycoplasma, safety

Viral and Viral Diseases
PCV2

PO-PT2-273

FIELD EFFICACY OF A NEW READY TO USE VACCINE PORCILIS® PCV MHYO vs A VACCINE PROGRAM WITH PORCILIS® PCV AND M+PAC

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Introduction: Piglets are quite frequently vaccinated against both *M.hyopneumoniae* (M.hyo) and PCV2, two of the most prevalent pathogens in finishing pigs. The majority of commercial vaccines are monovalent products that require one or two vaccinations or have to be mixed before injection. Therefore, a safe and efficacious ready-to-use one dose combination product that is convenient for the user and reduces the number of injections given to piglets is highly desirable. This study reports the results obtained following comparison of efficacy of a new one dose combination product Porcilis® PCV M Hyo against Porcilis® PCV and M+PAC applied separately on the same day.
Materials and Methods: The study was conducted in a farm in Southern Spain (Murcia) with three phase production and 2100 sows, positive for PRRSv, Mycoplasma and PCV2. Performance results were reviewed in the study (ADG and FC, standardized 18-105 kg) for all pigs produced from January until July 2015 (32 869 pigs), in different fattening units. Piglets were vaccinated at 3 weeks of age with Porcilis PCV and M+PAC, except for 4449 piglets that were vaccinated with Porcilis PCV M Hyo (PM) at 3 weeks of age in June and July and compared to 5347 piglets as control group (C) vaccinated with standard PCV and M+Pac (P+M) program in pre- and post-trial fattening rounds. Lung lesions were scored on a scale from 0 to 5 (Bollo score) in control and test groups between October and November.
Results: The piglets entered the fattening units with an average weight of 23.94 kg and went to slaughter at 102.2 kg. Pigs fattened from January until the trial (June-July) had ADG = 584g and FC = 2.44. Pigs in control group (P+M) from previous fattening rounds near PM group (3750 pigs) had ADG = 662g and FC = 2.35, while pigs post-trial (1597) ADG = 611g and FC = 2.45. The PM pigs that arrived in fattening units in June-July, had ADG = 672 g and FC = 2.29 ($p>0.05$ compared to C). The percent pigs (P+M=880 and PM=1060 lungs) with some lung lesions were 2.5 % in P+M vs 2.6% PM. No pigs had the highest lung lesion score (= 5) while the average score (Total of lung scores/Total No. of lungs) was 0.03 in PM and P+M group ($p>0.05$).
Conclusion: Porcilis® PCV M Hyo was safe and efficacious against PCV2 and/or M.hyo infections under field conditions and had similar results compared to vaccination with two single vaccines (i.e. Porcilis PCV and M+PAC).

Disclosure of Interest: None Declared

Keywords: Circovirus, Mycoplasma, convenience

Poster Abstracts

Viral and Viral Diseases
PCV2

PO-PT2-261

Comparison of three vaccination schemes against PCV2 and M. hyopneumoniae

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Introduction: Mycoplasma hyopneumoniae (M hyo) and Porcine Circovirus type 2 (PCV2) are of high economic impact in the swine industry. To select a vaccination scheme against both pathogens, the following points will be taken into consideration by the veterinarians and the farmers: efficacy, safety, ease of use. The aim of this side by side study was to compare the impact of 3 vaccination programs on piglet's performance shortly after vaccination and at slaughter.
Materials and Methods: The trial was conducted in a French farrow to finish 1200 sows PRRSv negative farm. In total, 1625 piglets of 3 following farrowing batches were included into the study. One day before weaning, piglets within each litter were alternatingly and randomly allocated to each of the 3 treatment groups, weighed individually and marked with an ear tag. Piglets in Group 1 were vaccinated with Ingelvac CircoFLEX® and Ingelvac MycoFLEX® freshly mixed (FLEXCombo®) using classical needles (1 ml each vaccine, IM). Piglets in Group 2 were vaccinated with Ingelvac CircoFLEX® and Ingelvac MycoFLEX® freshly mixed (FLEXCombo®) using a needle free device (1 ml each vaccine, IM). Piglets in Group 3 were vaccinated with Porcilis® PCV (2 ml, IM) and Porcilis® M hyo ID Once (0.2 ml, ID). In addition, 10 non-vaccinated sentinel piglets per batch were included to assess the PCV2 and M hyo infection. The piglets were weighed again individually 14 days after vaccination. Average Daily Gain at slaughter was calculated considering the number of days to slaughter.
Results: At inclusion mean body weights, sex ratio, parity as well as age were similar between the three treatment groups. The infection with PCV2 and Mhyo was confirmed for the sentinel animals. In total, 46 pigs died during the study: 18 in Group 1, 14 in Groups 2 and 3. 14 days after vaccination, the ADG was significantly higher ($p<0.001$) in both groups vaccinated with FLEXCombo® (Group 1: 225.99 g/day, Group 2: 225.52 g/day) compared to the group vaccinated with Porcilis® PCV and Porcilis® Mhyo ID Once (Group 3: 206.9 g/day). At slaughter, the ADG was 699.69 g/day, 703.29 g/day and 693.35 g/day in Group 1, Group 2 and Group 3 respectively. The difference between Group 2 and 3 was still significant ($p<0.05$).
Conclusion: In this study, the performance of pigs that were vaccinated with FLEXcombo®, either administered via needles or with a needle-free device, was higher compared to pigs that were vaccinated with Porcilis® PCV and Porcilis® M hyo ID Once. FLEXCombo® is not only easy to use (a single injection to control 2 pathogens) but also safe and efficacious through the whole fattening period.

Disclosure of Interest: None Declared

Keywords: performance, Vaccination PCV2-Mycoplasma

Viral and Viral Diseases
PCV2

PO-PT2-229

Effects of two different circovirus type 2 and Mycoplasma Hyopneumoniae vaccination protocols on acute phase proteins in piglets

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Introduction: Vaccination against Circovirus type 2 (PCV2) and *Mycoplasma Hyopneumoniae* (M.Hyo) are applied round weaning. This is a critical period for this reason is very important applying vaccines that do not hinder adaptation to this new situation. The acute phase proteins (APPs) in serum have been proposed as suitable veterinary biomarkers to monitor the stress and the inflammatory response, which makes APPs notable parameters for the global assessment of pig welfare. The aim of this study was to evaluate the welfare to vaccination after the application of two different vaccination protocols, against PCV2 and M.Hyo, through of measurement of rectal temperature and two APPs; haptoglobin (Hp) and C-reactive protein (CRP).
Materials and Methods: In this study forty piglets Pietrain x (Landrace x Large White) crossbred, of 3.5 weeks of age, from a farm located in South-Eastern Spain, were used. The animals were divided in two groups of 20 animals (10 females and 10 males). The group A was vaccinated with 1 mL of CircoFLEX® and with 1 mL of MycoFLEX® in a single injection of 2 mL (FLEXcombo®; Boehringer Ingelheim, Spain, SA). The group B was vaccinated with Porcilis PCV® (Intervet International, The Netherlands) and Stellamune Mycoplasma® (Elanco Animal Heath, Spain) in two injections of 2mls each. Blood samples were taken before vaccination (baseline), 24 h post-vaccination (Post-V) and 48 h Post-V. Body temperature was measured before vaccination (baseline) and 8 h Post-V. The levels of Hp and CRP were measured using an automated biochemistry analyser (Olympus 2700, Germany). The statistical analyses were performed using GraphPad Prism 6 (Graph Pad, Sowfware, USA). A two-ways ANOVA test was performed and a value of $P<0.05$ was used to indicate significance.
Results: After 8 h Post-V, group B showed evidence of pyrexia relative to baseline ($P<0.001$) and rectal temperature levels in group B (40.6 ºC) were significantly greater compared to group A (39.7 ºC). The interaction between type of vaccination and day of sampling was significant for serum Hp and CRP. Group B had elevated concentration of Hp relative to baseline at 24 h Post-V and 48 h Post-V ($P<0.001$). Relative to baseline CRP concentration in group B were greater 24 h Post-V. Group B had significantly greater serum CRP concentrations compared to group A ($P<0.001$).
Conclusion: The lesser body temperature and production of APPs with FLEXcombo® contribute to welfare and may be facilitate the adaptation of piglets in this critical period. This enhanced adaptability of piglets vaccinated with CircoFLEX® and MycoFLEX® also have been showed in other trials.

Disclosure of Interest: None Declared

Keywords: acute phase proteins after vaccination, PCV2-Mycoplasma

Viral and Viral Diseases
PCV2

PO-PT2-018

Rapid visual detection of porcine circovirus 2 by loop-mediated isothermal amplification (LAMP) assay

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Introduction: Loop-mediated isothermal amplification (LAMP) is a novel nucleic acid amplification method in which reagents react under isothermal conditions with high specificity, efficiency and rapidity. Therefore, the main objective of this study was to develop a LAMP for rapid detection of PCV2. In addition, One of the most attractive features of this LAMP assay is that the results can be observed and determined by hydroxynaphthol blue (HNB) dye-mediated visualization using the naked eye and without opening the tubes after amplification.
Materials and Methods: Primer sets that could detect the PCV2 were designed. Nucleotide sequence data for PCV2 strains from the Genbank were aligned by using Clone Manager 6 to identify regions that equal between the genotype. Target ORF1~ORF2 gene specific primers were designed using a Primer Explorer V4 program on the Eiken website (http://primerexplorer.jp/e/). Six primers including outer primers (F3/B3), inner primers (FIP/BIP), and loop primers (LF/LB) for targeting ORF1~ORF2 genes. LAMP reaction mixture containing 1ul Bst DNA polymerase (8 U/ul, New England Biolabs, Ipswitch, MA, USA), 5ul template, 2.5ul dNTPs (10 mM), 8ul Betaine (250 mM), 1ul MgSO4 (150 mM), 1ul HNB (3mM, Lemongreen, Shanghai, China) and 1ul of each primer. To optimize of reaction condition for LAMP detection, the reactions were carried out at 61~ 65℃ for 60 min and performed the LAMP for different reaction times. The sensitivity of the LAMP assay was determined and compared with polymerase chain reaction and real-time PCR using the same template at identical concentrations.
Results: The PCV2 LAMP developed in this study was confirmed the detection of all of the PCV2. We chose 63℃ as the optimal reaction temperature. No amplification of product was found in 10 min, and positive amplified products were clearly detected after 40 min. The sensitivity of the PCV2 LAMP was confirmed that the same level or higher compared to the real-time PCR and PCR.
Conclusion: In this study, we developed a visual and rapid detection method for PCV2 using the optimized LAMP technique. Compared to conventional PCR analysis, the LAMP method has advantages such as time-saving, low cost and ease of operation. The LAMP extends previous methods for PCV2 detection and provides an alternative approach for detection of PCV2.The method is simple and obviates the need for expensive equipment such as real-time PCR instruments. So it is useful for clinical diagnosis in developing countries.

Disclosure of Interest: None Declared

Keywords: None

Viral and Viral Diseases
PCV2

PO-PT2-082

Molecular genetic analysis of porcine circovirus type2(PCV2) and PCVAD occurrence factors

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Introduction: Since 2005, PCVAD mainly caused by PCV2b had been a serious disease in Japan. After introduction of PCV2 vaccination in 2008, the PCV2 detection rate was drastically reduced and the disease was only observed in farms where the vaccination was not practiced or stopped. However, the detection has re-increased even on farms continuously vaccinated since about 2012 . We researched the re-emerging cases on field.
Materials and Methods: 1. Serums of five stages (30,60,90,120,150 days of age) collected from 17-24 farms for regular health check during 2008-2015 were analysed by qPCR.
2. qPCV2 positive serums(41samples) were served for genotyping by using either PCV2/DNA sequencing or RFLP method.
3. Vaccination scheme in terms of schedule, volume and product type, and health status were investigated in the farms showing PCV2 detection rate increase.
Results: 1 . In2008 and 2009, PCV2 was only detected in the later stages not vaccinated. In 2010 and 2011 there was no detection in almost stages and the number of positive farms did not increase. PCV2 apparently reemerged in terms of the detection rate and the viral quantity from 2012 onwards. In 2015, even young pigs of 60 days of age already showed an increase in viral quantity
2. The phylogenetic tree by ORF2 sequence of PCV2 and determind their genotypes.In 2008 ,PCV2a:8 PCV2b:1 PCV2a+b:1 PCV2d:0 2009,PCV2a:8 PCV2b:9 PCV2a+b: 1 PCV2d:0 2010,PCV2a:2 PCV2b:3 PCV2a+b:1 PCV2d:0 2015,PCV2a:4 PCV2b:0 PCV2a+b:0 PCV2d:3.ORF2 of detected PCV2d showed 99% homology with an American strain isolated after 2012 and showed 97% homology with Japanese strain isolated in 2008.
3. Through an interview among farms having shown PCV2 increase, three farms had errors in vaccination procedure(dose inadequacy and handling). One farm simultaneously suffered from PRRSv and two had PED outbreak. All the farms other than the first four mentioned above were infected by PCV2d. The two farms with PED increased PCV2 viral load prior to the PED outbreak and they had changed vaccination schedule and the type of vaccine.
Conclusion: There were multiple reasons for PCV2 increase. PCV2d was isolated in all farms except the ones with vaccination errors. PCV2 genotype confirmation is recommended when PCV2 viral load is increased, along with improvement in the vaccination method and health management. A strain closely related to mPCV2 isolated not only in Japan but also in US was confirmed. Whether an already-existed strain in Japan reemerged by its high proliferation ability, or it entered into the country recently, yet remains to be answered. Nevertheless, the timing and the route of emergence of PCV2d needs to be further investigated for comprehensive disease protection.

Disclosure of Interest: None Declared

Keywords: Genotype, occurrence factors, PCV2

Poster Abstracts

Viral and Viral Diseases
PCV2

PO-PT2-217

Production performance and Serological profile in a commercial farm in the Philippines vaccinated with Porcilis® PCV

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Introduction: In this study, we evaluated the production data and serological profile in a farrow to finish commercial farm unit that vaccinated against PCV2 in sows and piglets and off label sow vaccination at 4 weeks pre-farrow. Porcilis® PCV (MSD Animal Health) vaccine was administered as a comparative vaccine and production data of the finishers were measured. In consideration to possible maternally derived antibody interference, the piglet vaccination was scheduled at 5 weeks of age.
Materials and Methods: The farm was using Fostera PCV (Zoetis Animal Health) for more than 2 years already. Although production data was significantly better as compared to no PCV2 vaccination, they were interested in comparing the current performance against a new PCV2 vaccine in the market. Only the finisher data was evaluated and all data was compared at the end of the production phase. There were 180 pigs entered in the study; 90 piglets were vaccinated with Fostera PCV and 90 piglets were vaccinated with Porcilis PCV. The overall average values for the daily gain, mortality rate and serological profile (Biochek PCV, Optifarm Laboratory, Philippines) were analyzed by the number of pigs placed in each group. Serum samples were also analyzed in a PCV2 qPCR (MSD Boxmeer, The Netherlands). The data were analyzed using Mann-Whitney Test for comparison on a group basis.
Results: Porcilis PCV pigs averaged 98.67 kgs harvest weight compared to 93.92 kgs for Fostera PCV pigs. Porcilis PCV group had significantly higher weight gain (87.65 kgs vs 82.67 kgs Fostera PCV) and the average daily gain of 737 grams was 42 grams higher than Fostera PCV group.

The serum was tested using the Median Diagnostics VDPro PCV2 Ab ELISA Test Kit to measure the S/P ratio from the time of vaccination until 2 weeks before the animals were sold to market. The levels of antibodies remained consistently high until 21 weeks in pigs vaccinated with Porcilis PCV (from 1.744 S/P to 1.488 S/P). The level of antibodies in the Fostera PCV vaccinated animals started at 1.496 S/P and consistently declined to 0.704 S/P ratio at 17 weeks of age. At 21 weeks, following PCV2 field exposure, Fostera PCV pigs seroconverted to 1.892 S/P ratio, which was higher than the post vaccination level and coincided with a positive PCV2 qPCR.
Conclusion: In this study, Porcilis PCV vaccination of 5 week old pigs improved average daily gain, produced consistently higher serological response throughout the fattener production phase and prevented PCV2 viremia compared to Fostera PCV vaccination.

Disclosure of Interest: None Declared

Keywords: Porcilis PCV, Production Performance, serological Profile

Viral and Viral Diseases
PCV2

PO-PT2-214

Comparison of PCV2 vaccines in two commercial farms in the Philippines

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Introduction: In this study, we compared the production performance of PCV2 vaccinated animals in terms of weight gain, average daily gain and mortality in two commercial farms. One farm vaccinated with Circovac (Merial Animal Health) and the other with Fostera PCV (Zoetis Animal Health) at 3 weeks of age. Both of these vaccination programs were compared against Porcilis® PCV (MSD Animal Health). The farms, which are located in the northern part of Luzon, use conventional housing in farrow to finish operation.
Materials and Methods: One hundred eighty (180) pigs in each farm were included in the study and were divided into two groups. Vaccination was done at 3 weeks of age. In Farm A, 90 pigs were vaccinated with CircoVac 0.5 mL, IM and the other 90 pigs with Porcilis PCV 2 mL, IM. In Farm B, 90 pigs were vaccinated with Fostera PCV 2 mL, IM and the other 90 pigs with Porcilis PCV 2 mL, IM. Data set of parameters such as body weights and mortalities were obtained. Observation was done from 3 weeks of age until harvest time. Body weights were measured at time of vaccination and harvest. Mortalities were recorded during the whole duration of the study. The data were analyzed using Mann-Whitney Test for comparison on a group basis.
Results: Among the parameters, mortality rate were significantly lower ($P<0.05$) in Porcilis PCV (8%) vaccinated group in Farm B compared to Fostera PCV (14%) vaccinated group. However, this difference was not significant in Farm A compared to CircoVac (3% vs 6% Porcilis PCV) vaccinated group. For the ADG, no significant difference was observed in Farm A. However, there is a significant difference of 0.33 kgs found in Farm B. Porcilis PCV vaccinated group yielded 5.10 kgs higher harvest than Fostera PCV vaccinated group.
Conclusion: The study demonstrated that there were significant differences in mortality rates and in average daily gain in Farm B, while theses parameters were only numerically different in Farm A. The higher % mortality in Porcilis PCV pigs in Farm A could not be explained. In summary, Porcilis PCV vaccination at 3 weeks of age improved production parameters under the conditions tested.

Disclosure of Interest: None Declared

Keywords: Better Performance, Commercial farm, Porcilis PCV

Viral and Viral Diseases
PCV2

PO-PT2-265

Effects of two different Circovirus type 2 and Mycoplasma hyopneumoniae vaccine combinations on acute phase proteins in piglets

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Introduction: Vaccination against circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* (*M.hyo*) is done round weaning, which is one of the most stressful events in the pig's life. Therefore, vaccination should not contribute to compromised well-being to this new situation. Acute phase proteins (APPs) have been proposed as suitable biomarkers to monitor stress, for detection of inflammation and for monitoring the well-being of pigs. The aims of this study was to evaluate the development of haptoglobin (Hp) and C-reactive protein (CRP), rectal temperature and the average daily weight gain (ADWG) obtained after application of two different vaccination protocols against PCV2 and *M. hyo* in piglets, during the nursery phase.
Materials and Methods: Two groups of 20 piglets (10 males and 10 females per group) were vaccinated, 7 days after weaning, with 1 mL of CircoFLEX® and with 1 mL of MycoFLEX® in a single injection of 2 mL (A, FLEXcombo®; Boehringer Ingelheim, Spain, SA) or with a single injection (2 mL) of (B) Porcilis® PCV-M Hyo (Intervet International B.V., The Netherlands). Blood samples and weight of each animal were taken before vaccination, 24h after vaccination (24h Post-V) and 48h after vaccination (48h Post-V). Also, the weight at 39 days after vaccination was taken (39d Post-V). The rectal temperature was recorded before and 7h after immunization. The Hp and CRP concentrations in serum were determined using an automatic biochemical analyzer (Olympus 2700 automatic chemistry analyzer, Germany). The statistical analyses were performed using GraphPad Prism 6 (Graph Pad, Software, USA)
Results: The administration of both vaccines increased concentrations of Hp and CRP respect to basal level of each group. In addition, 24h Post-V, Hp and CRP concentrations were significantly higher (approximately 3-fold higher) in group B compared to group A. 7h post immunization, the rectal temperature was significantly higher ($p < 0.01$) in animals vaccinated with B (40.9 ºC) compared to A (39.9 ºC). Moreover, in relation to baseline, the ADWG were higher in animals vaccinated with A compared to the animals vaccinated with B at 24h (173g vs -29g) and at 48h (193g vs 48g) Post-V.
Finally, the average weight gain 39d Post-V was of 13kg for A and 11.5kg for B.
Conclusion: It has been reported that weaning stress increases serum level of APPs and that the Hp level and the ADWG may be inversely related post-weaning. In this study, significantly higher levels of both APPs in animals vaccinated with Porcilis® PCV-M Hyo compare to FLEXcombo® were found and the lower ADWG was obtained in the group with these highest levels of APPs.

Disclosure of Interest: None Declared

Keywords: acute phase proteins, post weaning weight, Vaccination PCV2-Mycoplasma

Viral and Viral Diseases
PCV2

PO-PT2-254

Reducing PCV2 viremia in neonatal pigs through sow mass vaccination in a unstable herd

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Introduction: *In utero* infection of piglets with PCV2 may serve as a potential source of PCV2 vertical transmission to the offspring[1]. This infection might make newborn pigs more susceptible to co-infections with other pathogens and therefore may be associated with PCVAD in the growing pig[2].
It has been shown before that Ingelvac CircoFLEX® is safe when used in sows [3].
The objective of this study was to determine the prevalence and viral load of PCV2 viremia in pre-suckle piglets before and after sow mass vaccinations.
Materials and Methods: This study was conducted in a 2800 sow herd located in Toledo, Spain. The herd is positive for PRRS, M. hyo. Pigs are vaccinated with CircoFLEX® weekly at 3 weeks of age. PCV2 was detected in 4 week old piglets showing performance problems during nursery phase although the sow herd didn´t have reproductive performance problems.
To evaluate the stability of the herd to PCV2, we bled 39 presuckle piglets and individually qPCRs were run following this protocol:
- 3 piglets per litter from 5 parity 1-2 sows(P1-2)
- 3 piglets per litter from 4 parity 3-4 sows(P3-4)
- 3 piglets per litter from 4 parity ≥ 5 sows.(>P5)

The same sampling was repeated 2 months after mass vaccination of the whole sow herd with Ingelvac CircoFLEX®(1ml).Another mass vaccination was applied due to high pressure of the virus.The third sampling was done 2 months after the second vaccination.
All statistical analyses were performed using SPSS v.15 (SPSS Inc. Chicago, IL, USA). Differences were considered statistically significant at $p<0.05$.
Results: In the first sampling corresponding to piglets from non-vaccinated sows, PCV2 virus was detected in 66,7% of the piglets in P1-2,70% in P3-4 and 33,33% in >P5 and the mean of viral load(expressed as log10) was high showing 8.87 in P1-2, 7.13 in P3-4 and 5.66 in >p5. When looking at the different parity groups, after the first mass vaccination the results didn´t show any difference of PCV2 positive piglets but a strong reduction was observed in viral load (8,87 vs 3.74 in P1-2, 7.13 vs 4.73 in P3-4 and 5.66 vs 4.31 in>P5).None of the samples after the second mass vaccination resulted in positive sample, reducing the viral load below the detection level.
Conclusion: According to the results obtained by qPCRs, we can conclude that is possible to reduce prevalence and viral load of PCV2 in presuckle piglets by using Ingelvac CircoFLEX® in a mass vaccination protocol in sows.
Further studies are necessary to determine whether there is a correlation between prevalence of PCV2 in presuckles pigs and performance parameters.

Disclosure of Interest: None Declared

Keywords: unestable PCV2 herd, Vaccination PCV2 in sows

Poster Abstracts

Viral and Viral Diseases
PCV2

PO-PT2-009

A rapid and simple molecular diagnostic test for penside detection of Porcine circovirus (PCV2)

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Introduction: Porcine circovirus type 2 (PCV2) infections in pigs is a major challenge for the swine industry as it causes significant production and economic losses to producers worldwide. Timely detection of PCV2 in herds is important in order to minimize the spread of infection and reduce economic losses. Current diagnostic methods such as ELISA and PCR are not suitable for field use because of the need for expensive equipment, trained technicians, and a specialized laboratory. To address this unmet need, we have developed an easy to use “sample-to-answer” molecular diagnostic test for penside detection of PCV2 directly from oral fluid samples.
Materials and Methods: This assay is based on loop mediated isothermal amplification (LAMP) amplification of ORF2 gene. For amplification, primers are mixed with an isothermal buffer, fluorescent dye, and target, followed by incubation for 40 minutes under isothermal conditions. Initially, serial 10-fold dilutions of PCV2 genomic DNA were tested to determine sensitivity in a clean system. Fluorescent dye in the reaction mixture allowed real-time monitoring of amplification products and post reaction thermal melt analysis to confirm the correct amplification product. Furthermore, complete formulation (buffer, enzyme, dye, and primers) sufficient for single reactions were lyophilized in 0.2 ml PCR tubes. An easy to use sample preparation method, which involves dilution of sample (oral fluid) in a lysis buffer followed by incubation at 90°C for 5 min., was also developed. The performance of this sample preparation method was evaluated by testing oral fluid samples spiked with different titers of PCV2 virus. To determine the clinical performance of this method, 20 samples (12 positive and 8 negatives) were tested.
Results: The analytical sensitivity of the assay using purified DNA was demonstrated to be about 30 copies of DNA, which is comparable to real time PCR. Using the newly developed sample preparation method, a sensitivity of 500 virus/test within 40 minutes was achieved. When tested using clinical samples, the sensitivity and specificity of this assay were found to be 89% and 100%, respectively.
Conclusion: Results indicate that this new LAMP assay is a rapid and effective method for detection of PCV2 directly from oral fluid samples. This assay can be run on a simple and easy to use, portable isothermal amplification unit and results are avilble within 40 minutes, making it ideal for penside diagnostic testing.

Disclosure of Interest: B. Converse Conflict with: Lucigen corp, J. Benzine Conflict with: Lucigen corp, K. Brown Conflict with: Lucigen corp, K. Yoon: None Declared, S. Goyal: None Declared, D. Mead Conflict with: Lucigen corp, Y. Chander Conflict with: Lucigen corp

Keywords: Isothermal amplification, Molecular diagnostics, Porcine Circovirus 2

Viral and Viral Diseases
PCV2

PO-PT2-200

Phylogenetic analysis of porcine circovirus type 2 (PCV2) strains in Northern Ireland from current and archival samples.

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Introduction: Porcine circovirus type 2 (PCV2) is the causative agent of the post weaning multisystemic wasting syndrome (PMWS). PMWS is a damaging disease that affected the pig industry worldwide. In the UK it was estimated to the have cost the industry £88 million during the epidemic period before the disease was controlled by vaccination. PCV2 can be divided into four genotypes known as PCV2a, b, c and d. It has been reported in several countries that a shift of prevalence from PCV2b to PCV2d is ongoing. Some reports of PCV2d associated wasting disease in vaccinated animal have been published but any role of PCV2d in vaccine failure needs further research.
For almost a decade, no phylogenetic study has been published on PCV2 strains circulating within Northern Ireland (NI) pig herds. The objectives of this study was to carry out a phylogenetic evaluation of the currently circulating strains of PCV2 in Northern Ireland and to detect if the PCV2d was present in the NI pig herds. Current strains were also compared to archival sequences from the Island of Ireland in order to estimate shifts in genotype.

Materials and Methods: Twenty two PCV2 positive mesenteric lymph node (MLN) samples were collected between 2011 and 2015. The open reading frame 2 (ORF2) gene, coding for the viral capsid, was amplified by PCR. Gel bands were excised, cleaned and sequenced by standard methods. Sequences were processed using Geneious software and phylogenetic trees were produced using Mega5. The sequencing data was analysed with an additional 28 archival sequences dating from 1997 to 2006 collected prior to the introduction of PCV2 vaccination.
Results: All sequences identified in this study were PCV2a or PCV2b. In total PCV2a accounted for 28% (14/50) of sequences and PCV2b for 72% (36/50) genotypes. The analysis has show a shift way from PCV2a to PCV2b. Among archival sequences 35.7% (10/28) of sequences were PCV2a while 64.3% (18/28) were PCV2b. In currently circulating strains 18.2% (4/22) were PCV2a and 81.8% (18/22) were PCV2b.
Conclusion: Sequence analysis revealed increased percentage of PCV2b strains in current samples compared to archival. Although only a small number of sequences have been analysed, it is likely to conclude that PCV2b genotype is predominant in Northern Ireland. This finding is consistent with previous study which confirms that genotype shift in NI occurred around 2003. There is no evidence of the genotypes PCV2c or d found in NI pig herds but close monitoring of the evolution of this virus is important, particularly if selective pressures due to vaccination are producing new strains that are capable of causing disease in vaccinated animals.

Disclosure of Interest: None Declared

Keywords: PCV2, PCV2 vaccine, PCV2d

Viral and Viral Diseases
PCV2

PO-PT2-203

Characterization of specific antigenic epitopes and the nuclear export signal of the porcine circovirus 2 ORF3 protein

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Introduction: *Porcine circovirus* 2 (PCV2) is the etiological agent of postweaning multisystemic wasting syndrome. PCV2 ORF3 protein is a nonstructural protein known to induce apoptosis, but little is known about the biological function of ORF3 protein. Therefore, we undertook this study to map ORF3 protein epitopes, characterize putative nuclear localization (NLS) and nuclear export (NES) sequences in ORF3, compare Virus kinetics and animal pathogenicity between wild type PCV2 and ORF3-deficient PCV2 (mPCV2).
Materials and Methods: In the present study, recombinant GST-ORF3 and his-ORF3 proteins were expressed in E. coli and mAbs were produced by these two fused proteins, peptide-ELISA and peptide-dot-blot, ELISA additivity test, western blot and Indirect immunofluorescence assay (IFA) analysis were carried out to analyze the epitopes targeted by these mAbs. Recombinant plasmids containing putative NLS, NES, mutated NES were transfected into PK15 cells and confocal microscopy were used to analyze the function of NLS and NES in ORF3. Localization of ORF3 in PK15 cells is analyzed by both infection and transfection forms. Virus kinetics and animal pathogenicity test of PCV2 and mPCV2 were tested in PK15 cells and in Balb/c mice, respectively.
Results: mAbs 3B1, 1H3 were generated against GST-ORF3 and mAb 3C3 was generated against his-ORF3. We find that ORF3 in PCV2 infected cells contains a conformational epitope targeted by the antibody 3C3, which is distinct from linear epitopes recognized by the antibodies 3B1 and 1H3 in recombinant ORF3 protein. These results suggest that the linear epitope recognized by 3B1 and 1H3 is masked in PCV2 infected cells, and that the conformational epitope is unique to PCV2 infection. Furthermore, we find that ORF3 protein expressed in cytoplasm in early stages of PCV2 infection and then accumulated in nucleus over time. Moreover, we localize a NES at the N-terminus (residues 1-35aa) of ORF3 which plays critical role in nuclear export activity. PCV2 and mPCV2 were rescued by transfection of infectious clones, the growth curves of PCV2 and mPCV2 show that there was no significant difference between these two viruses (P>0.05), indicating that the ORF3 protein is not required for replication in cell culture. There was no significant differences between PCV2 and mPCV2 groups in viremia and induced antibody levels in animal experiment, but the wild type PCV2 can cause more obvious pathological changes of spleen than ORF3-deficient PCV2, indicating that ORF3 may related to pathogenicity of PCV2.
Conclusion: These findings provide a novel insight that deepens our understanding of the biological function of PCV2 ORF3.

Disclosure of Interest: None Declared

Keywords: None

Viral and Viral Diseases
PCV2

PO-PT2-302

The use of ELISA and Real Time PCR for the detection of PCV2

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Introduction: The interpretation of the ELISA or PCR results for PCV2 detection is often difficult. The aim of the study was to compare PCV2 seroconversion, viremia, shedding in feces and presence in oral fluid in three Polish farms.
Materials and Methods: The serum, feces and oral fluid samples were obtained from two, two site farms with low level of biosecurity and hygiene (farm 1 and farm 2), and from one farrow to finish farm with very high biosecurity level and hygiene (farm 3). In farm 2 and 3 piglets at 3 weeks of age were vaccinated against PCV2 with CircoFLEX (Boehringer Ingelheim). The samples were obtained from 3 (except for farm 2), 6, 9, 12, 15, 18 and 21 week old pigs and sows (except for farm 2). From each age group 8-10 serum samples were obtained, as well as feces samples from the bled pigs. Also one oral fluid sample was collected per pen of weaners and fatteners. In three weeks old piglets oral fluid was collected individually with a cotton swab. Serum samples were analysed with Ingezim Circovirus IgG/IgM ELISA (Ingenasa). Serum, feces and oral fluid samples were analysed with in house Real Time PCR for PCV2.
Results: In farm 1, despite no vaccination against PCV2, the nursery population seemed free from the infection until 9 weeks of age. PCV2 was detected in serum, feces and oral fluid at site 2, in all animals from 12 to 21 weeks of age, as well as IgG and IgM seroconversion in majority of fatteners.
In farm 2 PCV2 in serum and feces was detected respectively in 2 and 12, out of 20 weaners, as well as in oral fluid. At site 2 of farm 2 viremia was less common, and Ct values higher, than at the site 2 of farm 1, where pigs were not vaccinated (52.5% vs 97.5% of viremic pigs, in farm 2 and farm 1 respectively). Despite infection of weaners and fatteners in farm 2, only 3 out of 60 serum samples were IgM positive.
In farm 3 viremia was detected in one sow and in oral fluid of 12 weeks old fatteners. All other samples were negative in Real Time PCR. Seroconversion with IgG was present in sows and 3 to 12 weeks old pigs. The result of PCV2 presence at low level in 2 out of 128 samples of serum and feces and 15 oral fluids, is difficult to interpret.
Conclusion: In summary, serological and Real Time PCR results for PCV2 have to be interpreted with care. Real Time PCR proves to be the best method for evaluation of PCV2 circulation. Vaccination seems to limit PCV2 viremia to a larger extent than its shedding in feces. Considering the ease of sampling and the results of this study, feces or oral fluid should be considered as best samples for Real Time PCR monitoring of PCV2, especially in vaccinated farms

Disclosure of Interest: None Declared

Keywords: PCV2, oral fluid, diagnostics

Poster Abstracts

Viral and Viral Diseases
PCV2

PO-PT2-239

Genetic characterization of Porcine Circovirus 2 field isolates from Italian farms

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Introduction: Porcine Circovirus Type 2 (PCV2) is an important pathogen related to several disease syndromes in pigs, collectively named PCVD (PCV disease). PCV2 strains are currently classified into four genotypes: PCV2a, PCV2b, PCV2c and PCV2d. The latter is considered an emergent genotype. It has been, indeed, increasingly isolated worldwide, mainly in cases of suspected vaccine failure, rising concerns about vaccine protection and possible ongoing genetic shift.

Materials and Methods:
In order to study the circulation of PCV2 strains related to PCVD outbreaks in Italian farms, 46 samples with high viral loads, were completely sequenced. Samples had been conferred, between January 2014 and October 2015, to IZSLER diagnostic laboratories from 35 different herds. All the farms except 2 declared to apply a vaccination program against PCV2, and were recording an increase in clinical cases compatible with PCVD. PCV2 detection and quantification were performed by Real-Time PCR. PCV2 full-length genome sequence was achieved by Sanger method from tissue samples with more than 10 exp 8 genome copies/g or sera and oral fluids with PCV2 loads higher than 10 exp 6 genome copies/mL. Phylogenetic analysis was accomplished using the distance-based Neighbor-Joining method.

Results: PCV2a was found in 4 samples, from 2 (5,7%) vaccinated herds. PCV2b was the prevalent genotype and was identified in 24 samples from 21 farms (60%); only one was not vaccinated. The emergent genotype PCV2d, was detected in 15 samples from 10 herds (28,6%); only 1 had suspended the vaccination program. Finally, 3 samples from 2 vaccinated farms (5,7%) had mixed sequences of genotypes PCV2b and PCV2d. Considering the distribution of genotypes over the time, a substantial increase of PCV2d circulation in Italy during 2015 can be observed. Indeed, in 2014, among 21 investigated herds, 2 were infected by PCV2a, 21 (85,7%) by PCV2b and only 1 by PCV2d. On the contrary, in 2015 none of the investigated herds had PCV2a, PCV2b was recovered in 3 (21.43%), 2 had a concomitant circulation of PCV2d and PCV2b, while 10 (64.2%) were infected by PCV2d.
Conclusion: Data strengthen the hypothesis of PCV2d as an emergent genotype. Besides, the considerable increase in proportion of PCV2d infected herds recorded in this study in 2015, suggests the existence of an ongoing genetic shift between PCV2b and PCV2d. The role of vaccination pressure and natural selection is not clear yet and should be further investigated. Supporting diagnostic data with sequence analysis is extremely important in this context.

Disclosure of Interest: None Declared

Keywords: Genetic variability, Genotypes, PCV2

Viral and Viral Diseases
PCV2

PO-PT2-269

Comparing PCV2 piglet vaccines on several Dutch farms

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Introduction: Porcine circovirus (associated) diseases (PCV(A)D) are one of the main health problems in modern porcine industry. The disease has become endemic, ubiquitous and can nowadays be seen with milder clinical signs than in the past, from severe to unapparent, from the weaning age to the finisher stage and in the gilts and sows. Several commercial PCV2 vaccines are used in piglets in production to reduce or eliminate the clinical problems. The present paper reports a large field trial on 8 farms comparing the results from different piglet vaccines against PCV2.
Materials and Methods: On 8 different Dutch farms PCVD was controlled in the piglets with several different strategies. Overall 3 different commercial vaccines were used. On the 8 farms pre-Circovac (PC) data were compared with Circovac (CV) data, in most cases both periods for 1 year, depending on the farm data available. 5 farms used Circoflex, 2 farms Suvaxyn PCV and 1 farm Porcilis PCV. CIRCOVAC® (Merial, Lyon, France) was introduced in the piglet herds at or around weaning, 0.5 mL by intramuscular (IM) injection.
Technical data from the finishers were collected and compared, from 10 WOA, 25 kg till 117 kg live weight: average daily weight gain (Corrected Daily Growth), mortality and feed conversion (Energy Value conversion). The economical improvements were calculated on the basis of the Dutch economical values “Waarderingsnormen vleesvarkens 2013” of the Agricultural University of Wageningen.
Results: The number of sows on the 8 farms ranged from 105 – 900 sows, in total 4.180 sows, on average 523.
From these farms in total 108.543 piglets were followed in time, 51.726 in PC and 56.817 in CV, with on average 5.747 piglets in the PC and 6.313 piglets in the CV groups.
Overall the average Corrected Daily Growth (25 till 117 kg) of the PC group was 794 ± 36 g/d and from the CV group 819 ± 43 g/d (p = 0.066). Energy Value Conversion was respectively 2.689 ± 0.097 and 2.674 ± 0.077 (p = 0.44). Mortality was respectively 2.85 ± 1.24 % and 2.14 ± 0.80 % (p = 0.06).
The average financial improvement of these 8 farms was calculated on € 17.797,-.
Conclusion: In this large field trial with 8 farrow-to-finish sow farms the overall technical data improved in the finishing pigs when changing from another commercial PCV2 piglet vaccine to Circovac 0.5 mL IM. The Corrected Daily Growth increased, the Energy Value Conversion decreased, both significantly. The Mortality was reduced, non-significantly. This resulted in an average economic improvement of € 17.797,- per farm as a result of the use of Circovac vaccination in piglets.

Disclosure of Interest: A. Verhaegen Conflict with: Merial B.V., V. Dekens Conflict with: Merial B.V., T. Meyns Conflict with: Merial N.V., H. Smits Conflict with: Merial B.V.

Keywords: vaccination

Viral and Viral Diseases
PCV2

PO-PT2-274

Seroprevalence of Porcine Circovirus type 2 (PCV-2) in Mexican Hairless Pigs in Yucatan, Mexico.

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Introduction: The Porcine Circovirus type 2 (PCV-2) has been reported as one of the most important pathogens in the swine production worldwide due to the economic losses that caused, mainly in piglets, in which cause Post Weaning Multisystemic Syndrome and Porcine Dermatitis and Nephropathy Syndrome. The seroprevalence of PCV-2 have been reported up to 100% in American pigs and up to 98.1% in backyard pigs. However, there is a lack of information on the status of PCV-2 in Mexican Hairless Pigs (MHP) in Mexico. For this reason, the objective of this study was to estimate the seroprevalence of PCV-2 in MHP piglets and its progenitors in Yucatan, Mexico.
Materials and Methods: Serum samples were collected from 162 MHP piglets 2-3 month-old from 12 farms. The majority of the studied piglets showed diarrhea, weight loss, respiratory problems and skin lesions (erythematous plaques covered by crusts). Additionally, 26 piglet´s progenitors were blood sampled. From the 162 piglet serum samples, 34 pools were obtained. Serum samples from progenitors and pools from piglets were tested by indirect ELISA (BioCheck®) to detect antibodies against PCV-2. Seropositive samples were analyzed by RT-PCR. In addition, 100 piglets were slaughtered and samples of kidney, lung, intestine, lymphnodes and spleen were taken for histopathological study.
Results: 94.1% of the piglet´s pools (32/34) were seropositive, and 97.0% (31/32) of seropositive pools were positive by RT-PCR. 92.3% (24/26) of the progenitors were seropositive, and 8.3% (2/24) of these were positive by RT-PCR. The most important histopathological findings were whitish foci in kidney, Peyer patches proliferated, not collapsed lungs with elastic consistency, spleen and lymphnodes with decreased size. All piglets showed at least one of the following lesions: interstitial lymphohistiocytic nephritis with tubular necrosis, ulcerative lymphohistiocytic ileitis with lymphoreticular atrophy of mucosa-associated lymphoid tissue, interstitial lymphohistiocytic pneumonia, lymphoreticular atrophy with splenitis and histiocytic lymphadenitis, suggestive with histological changes caused by PCV-2 in piglets.
Conclusion: We conclude that MHP piglets and its progenitors in Yucatan, Mexico have high seroprevalence of specific antibodies against PCV-2. Together with the high DNA detection of the PVC-2 and suggestive clinical symptoms and histological changes in piglets confirm the circulation of PCV-2 in MHPs in Yucatan, Mexico. We are very grateful to FESE for supporting this project (SIST-PROY: FMVZ-2014-0010).

Disclosure of Interest: None Declared

Keywords: Mexican Hairless Pig, Porcine circovirus type 2

Viral and Viral Diseases
PCV2

PO-PT2-211

Implementing a PCV2 piglet vaccination on a Dutch farm

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Introduction: Post Weaning Multisystemic Wasting Syndrome (PMWS) was first identified in Canada in the mid-90's. Since then it evolved to the worst disease of modern swine industry as a complex of PCV2 diseases i.e. Porcine Circovirus (associated) Diseases (PCV(A)D). It is now present in all parts of the world. PMWS is characterized by a sudden wasting in weaners. Later PCVD is more often seen in the finishers and as a reproductive problem.
The aim of the present paper is to report on a recent field case of the improvement of PCVD related problems when using CIRCOVAC® (Merial, Lyon, France) as a piglet vaccination.
Materials and Methods: In the south of the Netherlands on a farrow-to-finish farm of 300 sows 9 batches of piglets were alternately vaccinated with Circovac 0.5 mL IM or not vaccinated. The boars are not castrated.
5 batches of 240 non-vaccinated piglets (NV) were compared with 4 batches of 240 Circovac vaccinated piglets (CV). The technical results were compared for the batches going into finishing during the period of June 1st till July 29th 2015. The comparison per batch was done on the basis of: Average Daily Weight Gain (ADWG), mortality %, % of animals in the hospital pen, meat percentage, loin depth (mm) and meat quality score at slaughter. The economical improvements were calculated on the basis of the Dutch economical values "Waarderingsnormen vleesvarkens 2015" of the Agricultural University of Wageningen.
Results: Mid 2015 alternating 5 batches of 240 NV finishers (data of in total 1.161 pigs) were compared with 4 batches of 240 CV finishers (data of in total 936 pigs). On average the NV animals went into finishing at a weight of 27.34 kg and had a carcass weight of 92.80 kg. The CV animals resp. 27.03 kg and 94.02 kg.
Both groups NV and CV, boars and gilts, had on average 105 days in finishing.
On average the ADWG was resp. 862 g/d vs 886 g/d, the mortality 3.25% vs 2.50%, % of animals in the hospital pen 4.03% (45 animals) vs 1.74% (16 animals), a meat % of 60.1% vs 59.9%, a loin depth of 63.2 mm vs 63.9 mm and a % AA (highest) carcass quality 22,81% vs 36,67%, % A carcass quality 76,18% vs 62,58% and a % B (lowest) carcass quality 1,01% vs 0,75%.
For this small Dutch family farm the economical improvement was calculated to be (on ADWG and mortality improvement) € 11.363,- on a yearly basis.
Conclusion: In this case, on a Dutch 300 sow farrow-to-finish herd, the implementation of PCV2 vaccination in piglet using Circovac yielded a clear performance improvement regarding ADWG, mortality and % of animals in the hospital pen. At slaughter the Circovac groups had a better carcass quality. All these are leading to a good economic improvement.

Disclosure of Interest: W. van Herten: None Declared, A. Verhaegen Conflict with: Merial B.V., V. Dekens Conflict with: Merial B.V., T. Meyns Conflict with: Merial N.V., H. Smits Conflict with: Merial B.V.

Keywords: vaccination

Poster Abstracts

Viral and Viral Diseases
PCV2

PO-PT2-282

DISTRIBUTION OF PORCINE CIRCOVIRUS TYPE 2 AMONG PIGS WITH RESPIRATORY PATHOLOGY IN DIFFERENT GEOGRAPHICAL REGIONS OF UKRAINE DURING 2014-2015

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Introduction: Ukraine is a country with a high level of risk and transmits for new infectious diseases of pigs. Accordingly information by 2011-2013, (PCV2) was one of the most circulated pathological agent of pigs in Ukraine. However, against a background of large-scale vaccination, the character of its circulation (PCV2) could change. So, the aim of our study was to track this aspect and to fix geographic dependence of (PCV2) involvement in the development of respiratory disease of pigs. Previous researches was found that the widest circulation of the most frequent discovery in complex respiratory disease of pigs in the Northern, Central and Eastern parts and much lower levels of it in Southern and Western parts of Ukraine.
Materials and Methods: The analyzed material include 69 samples from pigs aged 45 to 145 days and was taken from the different geographical areas of Ukraine. Real–time PCR method was used for analyzing material on the presence of PCV2 genetic material in the tissue. For research were used the samples of lungs and per bronchial lymph nodes that were taken from pigs with external manifestations of respiratory disease, which were subjected to euthanasia. The external manifestations included coughing, sneezing, discharge from the nasal cavity, and symptoms of low weight gain. After selection, the materials were frozen with -67 (°C) and used for next biomolecular researches. In particular, DNA was isolated by semi-automatic station (LSA MAX VET) and used for the implementation of PCR in real time, using oligonucleotides and probe that can flanking the area of gene capsid protein ORF2. Realization of reactions and detection results performed on equipment of software Applied Biosystems.
Results: During analyzation of results it was established that in 33 (48%) samples from 69 the DNA of PCV2 was present. According to 36 (52%) samples of viral material was absent. Researches were held in 17 regions of Ukraine, 10 of them had positive results (Central, Eastern and Western parts of Ukraine)
Conclusion: During the last years and even decades the intensive of PCV2 worldwide vaccination was implemented. The same thing took place in Ukraine but the intensity of distribution of PCV2 still have a high level. Porcine Circovirus type 2 was and still is widespread pathogen in Ukraine that implicated in the development of a number of pathological syndromes, one of the them is a complex respiratory disease. That means that the applied vaccination has only a partial effect on and do not facilitate on reduce of the circulation of virus or on removal from the population of pigs. Therefore there is a need to find alternative methods to influence on the PCV2 to reduce its distribution, including biotechnology.

Disclosure of Interest: None Declared

Keywords: PCV-2, Porcine circovirus type 2, respiratory diseases

Viral and Viral Diseases
PCV2

PO-PT2-003

A commercial PCV2a based vaccine protects pigs against experimental PCV2d challenge and reduces transmission to naïve pigs

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Introduction: The recent emergence of PCV2d strains and linkage of PCV2d with cases of porcine circovirus associated disease (PCVAD) in vaccinated herds have raised concerns over reduced efficacy of PCV2a-based vaccines. This study aimed to test the efficacy of CIRCOVAC® (Merial, Lyon, France) against PCV2d challenge in a conventional pig model and to determine the ability of the vaccine to prevent PCV2 transmission to naïve contact pigs.
Materials and Methods: Forty pigs were randomly divided into 4 groups: negative controls (NEG), vaccine controls (VAC), PCV2-challenged pigs (PCV2d), and vaccinated and challenged pigs (VAC-PCV2d). Vaccination was done at 3 weeks of age using CIRCOVAC according to label instructions. PCV2d and VAC-PCV2d were challenged with PCV2d 28 days later. Necropsy was done 21 days post challenge (dpi). Blood was collected on a weekly basis for ELISA PCV2 serology and PCV2 DNA analysis by real-time PCR. PCV2 DNA was also quantified in fecal swabs and nasal swabs on 7, 14 and 21 dpi. After necropsy, lymphoid tissues were assessed for microscopic lesions and determination of amounts of PCV2 antigen by immunohistochemistry (IHC). A mean lymphoid depletion score and mean PCV2 IHC staining score were calculated. Twelve PCV2 naïve contact pigs were housed individually in a different facility and were exposed to pooled feces obtained from PCV2d and VAC-PCV2d groups at 21 dpi to determine infectivity of PCV2d present in challenged pigs. Contact pigs were sampled for presence of PCV2 DNA in blood, feces and nasal fluid at the time of exposure, 7 and 14 days later.
Results: Vaccinated pigs had a slow PCV2 antibody response and at 28 days post vaccination 3/9 VAC and 1/9 VAC-PCV2d were seropositive. NEG and VAC pigs remained negative for the duration of the study. All PCV2-challenged pigs became infected. Vaccination significantly reduced PCV2d viremia (VAC-PCV2d) at 14 and 21 dpi compared to non-vaccinated infected pigs (PCV2d). Vaccination also reduced PCV2d nasal shedding and fecal shedding. The group mean lymphoid score was 0.1±0.5 for the NEG group, 0.3±0.5 for the VAC group, 1.6±0.5 for the VAC-PCV2d group and 2.5±0.5 for the PCV2d group. Vaccination significantly reduced the mean PCV2 antigen load in lymph nodes in VAC-PCV2d pigs compared to PCV2d pigs. When transmission was examined, PCV2d present in feces in non-vaccinated pigs, but not in vaccinated pigs, was infectious to naïve contact pigs suggesting a reduction of PCV2d transmission by vaccination.
Conclusion: Under the conditions of this study, the PCV2a-based vaccine was effective in reducing PCV2d viremia, tissue load, shedding and transmission indicating that PCV2a vaccination is useful in herds were PCV2d has been demonstrated.

Disclosure of Interest: None Declared

Keywords: Experimental Infection, Porcine circovirus type 2, Vaccination

Viral and Viral Diseases
PCV2

PO-PT2-263

PCV2 shedding profiles in oral fluid on clinically and sub-clinically affected farms.

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Introduction:
Little is known about oral fluid qPCR for PCV2, viral load and threshold values associated to clinical or sub-clinical disease. The aim of this study was to evaluate the usefulness of OF-based surveillance of PCV2 shedding on farms with or without clinical or subclinical PCVD.

Materials and Methods:
Shedding of PCV2 in OF was monitored at 2-weekly intervals in six wean-to-finish all-in-all-out farms. Sampling commenced at arrival (28 days old), after being PCV2 vaccinated, and ended in the 21st week of age. Six pens (16-120 pigs/pen) were sampled on each occasion in each farm with a rope:pig ratio of 1/25. Samples were transported on chill-packs to the laboratory (SAC, Penicuik, Edinburgh) where DNA was extracted (MagMax™ Pathogen RNA/DNA kit, Thermo Fisher Scientific®) and analyzed by qPCR (LSI VetMAX™ Porcine Circovirus Type 2– Quantification, Thermo Fisher Scientific®). Complementary necropsy samples of lung or lymph nodes were submitted for histological and immunohistochemical evaluation.

Results:
Farms 1, 2, 3, and 4 reported low total mortality (2.7% – 3.7%). Wean-to-finish average daily gain (ADG) from ranged from 790 to 875 g/day. Maximum levels of PCV2 in OF were $<5x10^3$ PCV2 copies/mL and shedding was found on all farms at most timepoints in >1 of the six sampled pens. There was no evidence of PCV2 capsid protein detected by immunohistochemistry (IHC) in post mortem samples.
Farm 6 reported total mortality of 8%, low ADG (under 800 g/day against expected ≈830g/day) and PCV2 load exceeded $1x10^6$ copies/ml in most of pens at 11 weeks of age and ranged between 10^4 and 10^5 copies/mL until finishing age in all pens. In 4/14 pigs, an interstitial pneumonia associated with positive IHC labelling confirmed clinical PCVD in these animals.
Farm 5 reported low mortality (1.6%) and an ADG of 830 g/day, a lower gain than expected. PCV2 levels in OF were markedly higher in one pen compared to the other 5 pens from week 7 (10^5 copies/mL) through to week 21 ($10^{7.9}$ copies/mL). The other 5 pens presented values $<10^3$ copies/mL until week 19 when they rose to 10^4 copies/mL. A single focus of positive PCV2 labelling was found in the bronchial gland of one animal out of 2 tested.

Conclusion:
Levels of PCV2 detection in OF differed between three farm categories, namely those with no evidence of active PCVD, a farm affected by clinical PCVD, and a farm in which subclinical PCVD was suggested.
More studies are needed to determine the **sampling requirements and virus copy thresholds that could define a range of potential PCVD-associated scenarios**. This study demonstrates the potential **benefit of qPCR PCV2 testing in OF in commercial conditions to monitor PCV2 status** in farms.

Disclosure of Interest: J. Hernandez-Garcia Conflict with: Zoetis, Conflict with: University of Cambridge, N. Robben Conflict with: Thermo Fisher Scientific, D. Magnee Conflict with: Thermo Fisher Scientific, C. Pettit: None Declared, I. Dennis: None Declared, T. Eley: None Declared, H. M. Martineau: None Declared, D. Werling: None Declared, S. M. Kayes: None Declared, J. R. Thomson: None Declared, A. W. Tucker: None Declared

Keywords: Oral fluids, PCV2, qPCR

Poster Abstracts

Viral and Viral Diseases
PCV2

PO-PT2-300

Assessing sow herd PCV2 stability utilizing colostrum, placental umbilical cord serum and placental umbilical cord swabs

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Introduction: Sow herd instability has been diagnosed on numerous farms in the U.S. during diagnostic investigations. Vertical transmission of PCV2 has previously been described using placental umbilical cord serum (PUCS), swab of the umbilical cord, colostrum, presuckle serum and fetal tissues. With each there are drawbacks on time, labor, biosecurity, safety and animal handling. The goal of this project is a comparison between sensitivities of PUCS, swab of the PUC and colostrum samples.
Materials and Methods: Paired PUCS and colostrum (n=659) as well as unpaired PUCS (n=692) and colostrum (n=669) sample results from eight sow herds (A-H) were compared as a litter evaluation. Five of the eight sow herds (C-D,F-H) compared paired (n=162) PUCS and swabs and unpaired PUCS (430) and swabs (162). To collect PUCS, expelled placenta was inverted and 3-4 umbilical cords (attached to the placenta and not visibly contaminated) were milked into a single serum tube. A swab of the same umbilical cords was taken (Herds C-D,F-H). Colostrum (1-3ml) was manually milked into a snap cap tube. PCV2 TaqMan Real-time PCR (HMC, Ames, IA; detection limit of <3.5 genomic equivalents/reaction) was performed on individual PUCS (tested in triplicate), swabs and colostrum. A production health survey was conducted on all herds and a subset of sows from each herd were measured using PCV2 ORF2 ELISA (HMC).
Results: Utilizing PUCS as the gold standard, colostrum had 67% sensitivity (95% CI, 48-92) and swabs had a 72% sensitivity (95% CI 55-85%). For paired samples, percent positive ranged from 0-52% (PUCS), 0-48% (swabs) and 0-19% (colostrum). For unpaired samples herds G and H, PUCS had significantly more positives. There were no significant differences in low positive (0-4%) sow herds (A-F). Only four of six low positive herds had positive PUCS samples (B, D, E, F). There were no differences in SP ratios as measured by ELISA, regardless of PCV2 stability.
Conclusion: These diagnostic comparisons indicate that PUCS, colostrum and swabs could be utilized to determine PCV2 stability at a herd level. Based on the production health survey, the fewer positive samples than expected in farms A-F is likely due to the management (herd closure) and vaccination of incoming gilts. The results indicate PUCS is more sensitive than colostrum and swabs in high prevalence but not in low prevalence sow herds. There are negligible differences between PUCS and swabs in regards to cost, time, skill level and biosecurity to collect each sample. In an unknown herd, PUCS is recommended over the other methods. PUCS protocols to determine sow herd stability have been implemented in over 40 herds.

Disclosure of Interest: None Declared

Keywords: Colostrum, PUCS, Stability

Viral and Viral Diseases
PCV2

PO-PT2-285

Meaning of active PCV2 infection in herds routinely vaccinating against PCV2 for the occurrence of pleurisy at slaughter in farms suffering from PRDC

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Introduction: Besides being the causing agent of porcine circovirus diseases (PCVD) PCV2 is also known as a major pathogen contributing to the porcine respiratory disease complex (PRDC). Despite vaccination against PCV2, active infection can be detected in some pigs at fattening. The relevance of these finding in regard of contributing to PRDC is a consistently discussed issue in the veterinary practice. The present study was conducted to estimate the meaning of active PCV2 infection in pigs from farms routinely vaccinating against PCV2 on herd level.
Materials and Methods: 300 fattening pigs from 10 farms routinely vaccinating against PCV2 were enrolled to this investigation. Blood was collected at placement, mid and end of fattening. PRRSV (PCR, ELISA), SIV (ELISA incl. serotyping), *M. hyopneumoniae* (ELISA) and APP (ELISA incl. serotyping) where monitored additionally within the same farms. Pigs with active PCV2 infection were defined as either PCV2 viremic (q-PCR) or IgM positive (ELISA) pigs in the fatting phase. Lung checks at abattoir were conducted for each animal regarding the occurrence of pleurisy.
Results: Laboratory diagnostic investigations revealed that pigs were seropositive for APP (67.8 %), SIV (40.5 %), PRRSV (69.8 %) and *M. hyopneumoniae* (67.8 %) in different combinations within the study period. In total 21.6 % of the pigs were either PCV2 viremic (6.3 %, n= 19), PCV2-IgM positive (13.6 %, n=41) or both (1.6 %, n=5). Mean level of PCV2 viremia was $2.2*10^5$ genome copies / ml serum. In total 37 % of all lungs showed pleurisy at slaughter. Univariate analysis (chi^2 test) revealed a positive correlation between IgM positivity and the amount of lungs with pleurisy (r 0.172; p= 0.004; OR: 2.58) whereas PCV2-PCR positivity did not. Binary logistic regression model including all pathogens mentioned above revealed that only seropositivity for APP serotype 2 had a significant influence on the occurrence of pleurisy at slaughter (p=0.009; ExpB: 2.39, CI: ExpB: lower: 1.24 upper: 4.59) whereas the influence of IgM was not significant (p = 0.105 ExpB: 3.71; CI: ExpB: lower 0.76 upper: 18.2) when considering all other pathogens.
Conclusion: In total 21.6 % of the pigs showed laboratory diagnostic results indicating active PCV2 infection (PCV2 viremia, IgM positivity) but total amount of PCV2 viremic pigs was low. For this investigation neither PCV2 viremia nor IgM positivity had a significant influence on the occurrence of pleurisy at slaughter. Therefore individual animals showing PCV2 active infection despite PCV2-vaccination in this study do not significantly increase the amount of lungs showing pleurisy at slaughter on herd level.

Disclosure of Interest: None Declared

Keywords: PCV2, vaccination, pleurisy,

Viral and Viral Diseases
PCV2

PO-PT2-262

Dynamics of viral load and antibody titers of PCV2 in commercial swine farms not-affected by clinical PCV2 infection.

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Introduction: Porcine Circovirus type 2 (PCV2) is prevalent among commercial swine in Chile, causing subclinical infection (PCV SI). Diagnostic techniques have been implemented to detect PCV2 infections; however, interpretation of the results in a subclinical scenario is difficult. Also, there is limited information about the dynamics between PCV2 antibody response and viral load in PCV SI. The objective was to determine the variability of antibody response and viral load of PCV2 in farms with PCV SI.
Materials and Methods: Viral load and antibodies against PCV2 were measured in 11 commercial swine farms in Chile, where there was no evidence recent PCV2 clinical disease. In each farm, serum samples were collected from groups of 16 pigs at 3, 10 and 20 weeks. Antibody titers were obtained using SERELISA® PCV2 Ab Mono Blocking kit and viral load was calculated using quantitative PCR (qPCR). To measure variability of PCV2 antibodies and viral load explained by swine belonging to a particular farm, we estimated the intra class correlation coefficient (ICC).
Results: Intra farm ELISA positivity ranged from 0.13-0.94, 0.38-1.00, 0.56-1.00 at 3, 10 and 20 weeks respectively. PCR positivity at 3, 10 and 20 weeks was 0.45, 0.84, and 0.45 respectively. The variability of antibody titers was greatly explained by the farm. The ICC was higher at 3 and 10 weeks of age (ICC=0.57 and 0.68 respectively), compared to pigs at 20 weeks (0.34), meaning that there is a high correlation of PCV2 ELISA results within farms, especially at 3 and 10 weeks.
Viral load variability was explained at a greater extent by the farm, especially at week 10 (ICC=0.89), which was significantly higher ($p<0.05$) than variability measured at week 3 and 20 (ICC=0.72 and 0.63 respectively).
Conclusion: There is a high variability of antibody response and viral load between farms without clinical PVC2. The intra farm correlation of PCV2 antibody titers was higher at 3 and 10 weeks of age, compared to older pigs. Intra farm correlation of viral load was high, especially at week 10. Although the variability of the antibody response and viral load was explained by the farm, other factors such as vaccines, vaccination protocols and other productive factors should be consider regarding to better understand the dynamic of PCV2 in PCV SI farms.
Results from this study can help understand the dynamics of PCV2 antibody response and viral shedding in commercial swine farms, and to provide information to guide the interpretation in non-clinical PCV2 scenarios.
This study has been partially funded by FONDEF IT13I20021 and Zoetis

Disclosure of Interest: None Declared

Keywords: monitoring, PCV-2, Subclinical

Viral and Viral Diseases
PCV2

PO-PT2-299

SAFETY AND EFFICACY OF A NEWLY DEVELOPED READY TO USE PCV2 AND MHYO VACCCINE (PORCILIS® PCV M HYO) IN A FARM WITH RESPIRATORY DISORDERS

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Introduction: Porcilis® PCV M Hyo is a newly developed vaccine that provides double protection against PCV2 and *M.hyopneumoniae* in a one and single administration. The objective of this trial was to demonstrate the safety and efficacy of Porcilis® PCV M Hyo in a commercial farm with respiratory disorders.
Materials and Methods: The trial was designed as a controlled, randomized and blinded study in a Spanish 800 sow farm. PCV2 and Mhyo infection were previously confirmed by seroconversion, PCR and by presence of compatible lung lesions at slaughter (LLS). A total of 614 piglets (4 wks old) were randomly allocated into two groups: PM (n=307, vaccinated i.m with 2ml of Porcilis® PCV M Hyo) and Control (n=307). All piglets were individually ear tagged and weighed at 4, 9, 18 and 22 weeks of life. Blood samples were also taken at the same ages (20 piglets/group). Mortality until slaughter was also recorded. Lung lesions compatible with Mhyo infections were scored at slaughter following the Goodwin and Whittlestone method. Pleurisy was also recorded. Safety was assessed by recording local and systemic reactions post-vaccination. All data were statistically analyzed using ANOVA, Levene, Pearson Chi Square and Kruskall Wallis test.
Results: No local reactions were detected in either group. One animal from the PM group had a minor systemic reaction that disappeared without intervention. Body weights at 9 weeks were not statistically different between groups (PM 17,8kg vs C 18,2kg; p=0,2). At 22 weeks of age, animals from the PM group were 2,23 kg heavier than the control group (PCVM 75,58 kg vs C 73,35kg; p<0,001). Consequently, ADWG was 30g higher in the PM group during the fattening period. Mortality linked to respiratory disorders in nursery was 8,8% higher in control than PM group (PM 8,14% vs C 17,26%; p<0,001). During the study period a PRRSv outbreak occurred in the nursery that resulted in increased mortality rates. In the fattening unit, mortality was also 3,26% higher in the control group (PM 2,93% vs C 6,19%; p=0,06). LLS at slaughter was lower in PM group (PM 5,65 vs C 7,04). No differences were found in pleurisy lesions.
Conclusion: Porcilis® PCV M Hyo demonstrated to be safe and efficacious in reducing mortality and growth retardation linked to respiratory disorders in a farm with PCV2 and Mhyo infection. Being a ready to use product adds the advantage of reduced management. Therefore, Porcilis® PCV M Hyo is an efficacious and convenient tool to control respiratory disease in growing piglets.

Disclosure of Interest: None Declared

Keywords: Efficacy, PCV2 Mhyo, RTU vaccination

Poster Abstracts

Viral and Viral Diseases
PCV2

PO-PCO1-001

PCV2d continues to increase in the US swine population

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Introduction: PCV2a is the longest known PCV2 genotype and the current commercial vaccines contain PCV2a. In recent years PCV2b has been the main strain identified in field cases in the US. Since 2012, PCV2d has been associated with apparent PCV2 vaccine failures in the US. In a large study investigating PCV2 sequences from 2012-2013 in the US, 37% of all 143 sequences investigated were classified as PCV2d whereas PCV2a comprised 12.6% of the sequences and PCV2b was identified in 52.4% of all sequences. The objective of the present study was to determine the PCV2d prevalence in US pigs during 2014 and 2015.
Materials and Methods: A total of 215 ORF2 sequences were obtained through the Iowa State University VDL LIMS system and corresponded to client requests for further characterization of the PCV2 PCR positive samples. In addition, lung tissues were obtained from 578 randomly selected cases submitted to the Iowa State University Veterinary Diagnostic Laboratory from January through March 2015. The DNA of these tissues was extracted and subjected to PCV2 ORF1 real-time PCR. All ORF1 PCV2 PCR positive samples were further tested by a differential real-time PCR based on ORF2 and capable of differentiating PCV2a, PCV2b and PCV2d. Selected PCV2 positive samples were also sequenced.
Results: Among the ISU-VDL sequences, 20.7% (44/213) corresponded to PCV2a, 9.4% (20/213) corresponded to PCV2b, 68.1% (145/213) corresponded to PCV2d and 1.9% (4/213) corresponded to the newly identified genotype PCV2e. Among all 587 lung samples, PCV2 DNA was detected in 22.7% (133/587). Specifically, PCV2a was detected in 10.4% (14/133), PCV2b was detected in 27.8% (37/133) and PCV2d was detected in 70.7% (94/133) of the lung tissue samples. Eighty-five of the 133 samples were sequenced and the sequencing results agreed with the PCR results. When VDL sequence data and lung tissue data were combined, there were a total of 358 PCV2 positive samples and 16.2% (58/358) were PCV2a, 15.9% (15.9/358) were PCV2b, 66.8% (239/358) were PCV2d and 1.1% (4/358) were PCV2e.
Conclusion: Overall the results indicate that the incidence of PCV2d has continued to increase from the first observation period spanning 2012-2013 (37%) to the second observation period spanning 2014-2015 (66.8%) and that PCV2d appears to replace PCV2b. This further highlights that PCV2d may have some advantage over PCV2a and PCV2b in its ability to replicate in pigs. Perhaps this also highlights the need for updated PCV2 vaccines.

Disclosure of Interest: None Declared

Keywords: Genotypes, Porcine circovirus type 2, USA

Viral and Viral Diseases
PCV2

PO-PT2-130

Microtubules mediate nuclear trafficking of PCV2

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Introduction: Microtubule transport of Porcine Circovirus Type 2 from the periphery of the cell to the nucleus is essential for viral replication in early infection. How the microtubule is recruited to the viral cargo remains unclear. In this study, we observed that PCV2 trafficking is dependent on microtubule polymerization.
Materials and Methods:
Cell cultures and virus infection. The PK-15 cells were cultured in minimal essential medium(MEM; Life Technologies/Gibco, Carlsbad, CA). PCV2 strain HZ0201 was propagated in PK-15 cells.
Confocal microscopy. Cells grown on glass coverslips were fixed, permeabilized with 0.2% Triton X-100, and incubated at 4°C with primary antibodies overnight. Cells were then incubated with FITC-conjugated secondary antibodies (KPL) and/or Alexa Fluor 546-conjugated secondary antibodies (Invitrogen) at 37°C for 1 h. Cellular nuclei were stained with 10 g/ml DAPI for 5 min, cover slipped, and viewed with a LSM780 laser scanning confocal microscope. The primary antibodies used included rabbit anti-α-tubulin polyclonal antibody (pAb) (ab15246; Abcam), mouse anti-Cap mAb.
Results: Microtubules mediate nuclear trafficking of PCV2
It is known that microtubules function as superhighways to mediate the transport of various cargoes. Intracellularly invading viral particles were examined in the early stages of PCV2 infection. Dynamic analysis showed that no visible viral particles had adhered to the cell surface at 0.5 hpi and that viral particles began to enter at 1 hpi. At 3 hpi, a number of viral particles had entered the cytoplasm and were near the cell membrane, where they began minus-end trafficking. At 9 hpi, a number of viral particles had been transported closer to the perinuclear region, and at 12 hpi, numerous viral particles were localized to the microtubule organizing centers (MTOC) near the nucleus, although no PCV2 virions were present in the nucleus itself. At 15 hpi, PCV2 Cap protein was present in the nucleus and most PCV2virions were no longer in the cytoplasm, suggesting that the PCV2genome was being delivered to the nucleus and was beginning to replicate. Additionally, many cytoplasmic PCV2 virions were adjacent, distributed along and within microtubules. In contrast, when cells were pretreated with different concentrations of nocodazole, the viral particles remained near the PCV2-infected cell membrane at 9 hpi. This suggested that the intracellular transportation of PCV2 virions was inefficient in cells with a compromised microtubular network.
Conclusion: The PCV2 virions travel toward the nucleus in a microtubule-dependent manner.

Disclosure of Interest: None Declared

Keywords: microtubules, nuclear trafficking, Porcine Circovirus Type 2

Viral and Viral Diseases
PCV2

PO-PT2-034

Concurrent experimental porcine circovirus type 2 (PCV2) and porcine parvovirus type 2 (PPV2) infection results in severe systemic PCVAD

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Introduction: Several independent research studies described the presence of PPV2 in association with PCV2 outbreaks in recent years. Since the late 1990's, PPV1 has been demonstrated to enhance PCV2 infection towards clinical disease; however, little is known about PPV2 and its importance, if any, for pig production. The objective of this study was to concurrently infect pigs with PPV2 and PCV2 and compare virus shedding, clinical disease and lesions to pigs singularly infected with PPV2 or PCV2.
Materials and Methods: Thirty-one pigs from a PCV2 negative herd were selected and randomly assigned to 4 groups and rooms with 7-8 pigs each. At three weeks of age the pigs were sham inoculated (negative controls) or received PCV2b inoculum (PCV2 group), tissue homogenate from a colostrum-deprived, snatch farrowed pig exposed to PPV2 (PPV2 group), or both PCV2b and PPV2 tissue homogenate by intranasal and intramuscular routes (PCV2-PPV2 group). Blood, fecal and nasal swabs were collected once a week and all pigs were euthanized at 21 days post inoculation (dpi).
Results: Negative controls and PPV2 pigs remained negative for PCV2 throughout the study as determined by serology and PCR. Pigs infected with PCV2 became positive for PCV2 DNA in serum, fecal and nasal swabs by 7 dpi and remained positive for PCV2 DNA in all sample types until 21 dpi. One PCV2 and one PCV2-PPV2 pig seroconverted to PCV2 by 21 dpi. PPV2 DNA was not detected in any of negative controls or PCV2 pigs. PPV2 DNA was detected in serum of individual PPV2 and PCV2-PPV2 pigs until 21 dpi and in nasal and fecal swabs until 7 dpi confirming a successful challenge. Microscopic lesions were not seen in negative controls or PPV2 pigs while moderate PCV2-associated lymphoid lesions were seen in 3/7 PCV2 pigs. In contrast, moderate PCV2-associated lymphoid lesions were present in 5/8 of the PCV2-PPV2 pigs and in this group microscopic PCVAD (severe lymphoid lesions associated with high levels of PCV2 antigen) was identified in 2/8 pigs.
Conclusion: The results of this study indicate that PPV2 is capable of enhancing PCV2-associated lesions and tissue levels and should be considered another co-factor in the PCVAD complex.

Disclosure of Interest: None Declared

Keywords: Experimental Infection, Porcine Circovirus 2, porcine parvovirus 2

Viral and Viral Diseases
PCV2

PO-PT2-168

Placental Umbilical Cord Sampling for Porcine Circovirus Type 2 and the effects of pooling on sensitivity of polymerase chain reaction results

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Introduction: Detection of Porcine Circovirus Type 2 (PCV2) in individual Placental Umbilical Cord Serum (PUCS) samples has been shown to be comparable to other sample types (e.g., colostrum, pre-suckle serum, fetal tissue) to monitor PCV2 sow herd stability. However, testing of individual samples is costly, and a frequently used method to reduce testing costs is sample pooling. The purpose of this study was to estimate the impact of PUCS sample pooling on PCV2 polymerase chain reaction (PCR) detection sensitivity.
Materials and Methods: Ninety-three PCR results per pool size were needed to detect a 20% difference in sensitivity (i.e., 50% vs 70%) as significant at an alpha probability $\leq$0.05 and power $\geq$0.8. All samples were collected from midwest United States breeding herds. Expelled placental tissues were gathered, placed in a refrigerator, at least three umbilical cords per placenta were collected and expressed into a serum seperator tube to make a single placental sample. A PCV2 rtPCR (HMC, Ames, IA) was run on each aggregate placental sample. Positive placental samples with Cq values ranging from 29.77-39.96 were categorized into high virus concentration (Cq $\leq$ 34.41), middle virus concentration (Cq between 34.42 and 36.22) and low virus concentration (Cq $\geq$ 36.25). Using known placental sample results, pools consisting of one positive sample plus one negative sample (2:1), one positive sample plus two negative samples (3:1) and one positive sample plus four negative samples (5:1) were tested on PCV2 rtPCR. The experimental unit was the placental sample rtPCR result, and pools that contained a PCR positive placental sample was considered as the positive gold standard for calculating relative sensitivity.
Results: Pooling 2:1, 3:1 and 5:1 resulted in a relative sensitivity of 81.7%, 76.3%, and 63.4%, respectively. All pools containing high virus concentration (low Cq) samples were positive. Pools containing middle virus concentration (middle Cq) samples were 100%, 80.6% and 54.8% positive for 2:1, 3:1 and 5:1 pools, respectively. Pools containing low virus concentration (high Cq) samples were 45.2%, 48.4%, and 35.5% positive for 2:1, 3:1 and 5:1 pools, respectively.
Conclusion: Pooling of PUCS-based placental samples reduced diagnostic detection sensitivity of PCV2 by rtPCR. As pooling increased, detection rates decreased. Further, the higher the initial Cq, the lower the detection rate for the middle and high Cq groups, i.e., pooling one middle or high Cq positive placental sample with one or more negative samples decreased detection rate in all pool sizes. These results can be used as a basis for further development of an optimal sampling protocol.

Disclosure of Interest: None Declared

Keywords: Placental Umbilical Cord Serum, Pooling, PUCS

Poster Abstracts

Viral and Viral Diseases
PCV2

PO-PT2-078

Comparison of three in situ methods that targets nucleic acid to detect PCV2

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Introduction: Postweaning multisystemic wasting syndrome (PMWS) is still one of the major economic problems for the pig industry. To confirm PMWS, it is necessary to fulfil the following diagnostic criteria: 1) specific clinical and necropsy findings; 2) specific histologic lesion and 3) presence of moderate to significant amount of PCV2 by immunohistochemistry (IHC) or *in situ* hybridization (ISH). The fact that the virus is ubiquitous in pig populations worldwide, including healthy animals, makes PCR- or isolation-based methods non-specific for disease identification. Criterion 3 has a crucial value since it confirms PMWS, however PCV2 can be present in the host as a bystander with low virus loads; this renders ISH and IHC ineffective. The most reliable method for detecting virus under these circumstances is PCR, since the virus load is usually $<10^8$ copies of nucleic acid per g of total DNA. An even more sensitive variation on PCR-based detection of PCV2 and other swine viruses is *in situ* PCR (IS-PCR). Still rarely used in veterinary medicine, this technique offers tremendous potential.
Materials and Methods: For the study 10 paraffin blocks were selected, 2 originating from lymph nodes negative for PCV2 by PCR, 6 PCV2 positive lymph nodes by ISH and 2 fetal livers, negative by ISH and positive by PCR. Primers, cycling conditions and probes were designed to amplify and detect a 501bp region of PCV2 ORF1 as was previously described. For direct (d)IS-PCR amplicons were directly labeled with DIG. For indirect (i)IS-PCR and for ISH a 41 base length DIG probe was used. Cycling conditions were optimized for IS-PCR.
Results: In all of the 6 positive lymph nodes the three methods showed positive result. However there was clear difference in the intensity of the signal. The lowest was obtained with ISH while the highest was in slides where dIS-PCR was applied. In two fetal livers, both dIS-PCR and iIS-PCR were able to detect PCV2, dIS-PCR seemed to be more sensitive while ISH stayed negative. Negative lymph nodes were negative by all methods.
Conclusion: IS-PCR seems to be very specific and versatile diagnostic tool more sensitive than ISH. IS-PCR was able to detect low number of copies of nucleic acid in tissues. However IS-PCR cannot completely replace ISH since it seems that is not suitable for detection when nucleic acid is present in high copy numbers.

Disclosure of Interest: None Declared

Keywords: None

Viral and Viral Diseases
PCV2

PO-PT2-240

Monitoring of physiological responses in piglets to different vaccines against PCV2 and M. hyo

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Introduction: Porcine circovirus type 2 (PCV2) and *Mycoplasma hyoneumoniae* (M. hyo) have a high prevalence among swine production systems globally and are both considered to be primary pathogens with high economic importance. For that reason, vaccination protocols against these two pathogens have become standard practice in modern pig industry. The aim of this study was to investigate the physiological response of piglets to vaccination with two commercially available vaccines against PCV2 and M. hyo by means of acute phase proteins, body temperature and weight gain.
Materials and Methods: Overall 79 pigs out of one farrowing batch in a commercial herd in Germany were randomly assigned to two treatment groups. Group 1 (n=37) was vaccinated with 2 ml freshly prepared mixture of Ingelvac CircoFLEX® and Ingelvac MycoFLEX® (Boehringer Ingelheim Vetmedica GmbH). Group 2 (n=42) was vaccinated with 2 ml of Porcilis® PCV M hyo (Intervet Deutschland GmbH). Both vaccines were brought to room temperature before use and injected intramuscularly into the right neck muscles at 21 days of age. Body temperature was measured prior to vaccination and 6, 24 and 48 hours post vaccination in all study animals. A subset of 19 (group 1) and 20 (group 2) piglets was subjected to blood sampling prior to vaccination as well as 24 and 48 hours post vaccination for determination of the acute phase proteins haptoglobin and C-reactive protein. Body weight was measured in all animals at the day of vaccination and at the end of nursery period at 10 weeks of age. Statistical analyses were performed by two-way ANOVA.
Results: Both treatment groups showed an increase of acute phase proteins in serum as well as rectal temperature compared to basal levels. However, this increase in both acute phase proteins and temperature was much more pronounced in the group vaccinated with Porcilis® PCV M hyo and thereby significantly higher compared to the group vaccinated with the mixture of Ingelvac CircoFLEX® and Ingelvac MycoFLEX®. During nursery, Ingelvac CircoFLEX® and Ingelvac MycoFLEX® vaccinated pigs showed a 1.2 kg higher weight gain compared to the other vaccination protocol (17.4 kg±0.54 vs. 16.2 kg±0.60, $p>0.05$).
Conclusion: The development of a vaccine with perfect interaction of adjuvants, antigen, target species and indication can be challenging. Piglets are facing many stressors during the weaning phase and so the impact of vaccines on the organism is especially critical during this period. Beside efficacy, the effect of a vaccine on physiological parameters is therefore a relevant criteria to achieve optimal results.

Disclosure of Interest: None Declared

Keywords: acute phase proteins, piglets, vaccines

Viral and Viral Diseases
PCV2

PO-PT2-161

Comparison of serological response to vaccination with Suvaxyn PCV® using a conventional administration method and a needle-free administration method

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Introduction: The injection of medical products using the conventional method of syringe with attached needle may cause safety issues in pigs (abscesses, transmission of pathogens) and personnel (self-injection). The use of a needle-free device would allow overcoming these disadvantages. However, the efficacy of such devices has to be demonstrated to be at least equivalent to that conferred by the conventional method.
The objective of the study was to demonstrate that the serological response induced by Suvaxyn PCV is equivalent in animals in which the vaccine was administered using a conventional method (syringe+needle) and in animals in which the vaccine was administered using a needle free device. The selected needle free device was AcuShot™.
Materials and Methods: Fifty 3-week-old pigs were included in the study and divided into 2 groups of 25 pigs each. In one group the vaccine Suvaxyn PCV was given with a needle attached to a syringe, by IM route; in the other group the vaccine Suvaxyn PCV was given using the AcuShot device, by IM route. On days 0 (prior to vaccination), 21, 42 and 63, blood samples were collected. Serum samples were analyzed by PCV2 ELISA test to determine the S/P ratio. The transformed serology data was analyzed using a general linear repeated measured mixed model with fixed effects treatment, timepoint and treatment by timepoint and random effects litter and animal within litter and treatment. Pairwise treatment comparisons were made at each time point if the treatment or treatment by time point interaction effect was significant (P£0.10).
Results: Seroconversion to vaccination was demonstrated in both groups, independently of the administration route of the vaccine. After a transitional decrease in ELISA S/P ratios at Day 21 after vaccination, a clear increase in titers was observed at Day 42.
The statistical analysis revealed no significant differences in serological titers between groups at all time points tested.
Conclusion: The serological response induced by Suvaxyn PCV in animals in which the vaccine was administered using a conventional method (syringe+needle) and in animals in which the vaccine was administered using the needle free device AcuShot™ was equivalent. No differences attributable to the administration system were demonstrated.

Disclosure of Interest: None Declared

Keywords: AcuShot, Needle-free vaccination, Suvaxyn PCV

Viral and Viral Diseases
PCV2

PO-PT2-184

Screening for PCV2 infections via cross-sectional sampling of 146 pig herds in Belgium: an overview of qPCR results over a 3-year period (2013-2015)

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Introduction: PCV2 infections can play an important role in swine respiratory disease. A feasible way of screening for PCV2 infections at herd level is cross-sectional blood sampling and qPCR analysis on pools of serum samples. The present study describes the results from VirusCheck, a service tool that includes herd information and serological investigation for PCV2.
Materials and Methods: For each herd (n=146), blood samples were collected from pigs of 3 to 5 different age groups, ranging from 6 to 25 weeks (W) (5 samples per group). Samples of each group were pooled, analyzed via qPCR and then classified as negative (≤10^3 PCV2 copies/ml), moderate viral load (10^4–10^6 PCV2 copies/ml) or high viral load (≥10^7PCV2 copies/ml). Results are grouped by age: 6-10W, 11-15W, 16-20W and >20W of age. Herd information including clinical signs and vaccination strategy was recorded from 2015 onwards.
Results: A total of 146 PCV2 screenings are included in the study: 62 in 2013, 35 in 2014 and 49 in 2015. The prevalence of PCV2-positive herds (at least one PCV2-positive pool) was respectively 55% (2013), 40% (2014) and 67% (2015). The clinical history of almost all participating herds mentioned growth retardation and coughing. Overall, in 2015, the proportion of negative pools per group was 82% (6-10W), 57% (11-15W), 49% (16-20W) and 64% (>20W). A moderate viral load was detected in 15% (6-10W), 34% (11-15W), 40% (16-20W) and 36% (>20W) of the pools, whereas a high viral load was found in limited number of pools (respectively 3%, 9%, 11% and 0%). In previous analysis (2013-2014), a high viral load was more frequently found (18%, 11W-20W) and a moderate viral load less frequently (25%, 11-20W) When the data of 2015 were looked at separately for PCV2-vaccinated (12 herds) and non-vaccinated pigs (35 herds), the percentage of PCV2 positive pools were 7% (6-10W), 18% (11-15W), 20% (16-20W) and 14% (>20W) in PCV2-vaccinated pigs versus 22%, 50%, 59% and 43% respectively in non-vaccinated pigs. High viral loads were only detected in herds without vaccination.
Conclusion: PCV2 infections with a high viral load (≥10^7 PCV2 copies/ml) became less prevalent in 2015 compared to previous years. The 2015 data also supports a protective effect of PCV2 vaccination against viremia. In line with this, the observed trend towards reduced viral loads might be explained by a higher vaccination rate in 2015 compared to 2013-2014. This hypothesis needs further investigation.

Disclosure of Interest: None Declared

Keywords: PCV2, qPCR

Poster Abstracts

Viral and Viral Diseases
PCV2

PO-PT2-137

Porcilis® PCV ID vaccination concurrently administered with Porcilis® M Hyo ID Once reduces mortality in Hungarian field efficacy study

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Introduction: PCV2 is main cause of Porcine Circovirus Diseases. *M. hyopneumoniae* is the primary agent of enzootic pneumonia. Vaccination against both minimizes the economic impact resulting from infection. The objective of this study was to assess the efficacy of the intradermal administration of Porcilis® PCV ID given alone or concurrently with Porcilis® M Hyo ID Once, with emphasis on mortality. The study was carried out in a Hungarian pig herd with confirmed PCV2 and M. hyo infections.
Materials and Methods: A total of 1810 healthy, 18-24 day old suckling piglets were allocated randomly, within litters, to one of three treatment groups: 1) PCV - vaccinated intradermally with Porcilis® PCV ID, 2) PM - vaccinated intradermally with Porcilis® PCV ID and Porcilis® M Hyo ID Once concurrently, 3) Control - untreated.
The primary efficacy parameters were PCV2 viraemia, average daily weight gain (ADWG) during finishing and mortality. Secondary parameters were overall ADWG (from vaccination to slaughter), morbidity, and PCV2 faecal shedding.
To obtain the required data, the first 940 included pigs were weighed individually at admission, at the end of the nursery period, and before slaughter. Medication given to these pigs was recorded. All pigs that died during the study were examined to establish the cause of death and this mortality was assessed on all 1810 included animals. From ±40 animals per group blood samples and faecal swabs were taken at regular intervals for determination of the PCV2 viral load by qPCR and antibody titres against PCV2 and M.hyo in the serum by ELISA.
Results: The PCV and PM pigs had a significantly better weight gain than the Control group during the finishing period and overall (ANOVA $p<0.0001$). The differences with the Control group were 44.5 and 50.9 g/day for the finishing and 25.0 and 29.7 g/day for the overall period, respectively.
Mortality was significantly lower in PCV and PM pigs than in the Control group with 9% mortality for PCV and PM groups and 14% for the Control group.
PCV and PM pigs were significantly less viraemic than the Control pigs.
Morbidity and faecal shedding were not statistically different across treatments.
Conclusion: Based on the study results, intradermal vaccination with Porcilis® PCV ID given as a single dose, alone or concurrently with Porcilis® M Hyo ID Once reduced mortality and viremia as well as improved ADG under field conditions. In addition, intradermal vaccination with a needle free IDAL injector has several benefits over intramuscular vaccination, including, ease of application, reduced volume, no muscle damage due to needle breakage, less stress on animals and administrator.

Disclosure of Interest: None Declared

Keywords: intradermal vaccination, mortality, PCV2

Viral and Viral Diseases
PCV2

PO-PT2-162

Beneficial impact of vaccination with a PCV2 vaccine and a Mycoplasma hyopneumoniae bacterin in piglets under French field conditions

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Introduction: PCV2 and *Mycoplasma hyopneumoniae (M hyo)* are the etiologic agents of PCV2-Diseases (PCVD) and enzootic pneumonia (EP), respectively. They are both known to play an important role in porcine respiratory disease complex (PRDC). The objective of this study was top evaluate the benefit of adding a PCV2 vaccination to a regular *M hyo* vaccination on standard production performance and EP lung lesions.
Materials and Methods: The trial was conducted in a PRRSv-negative farrow-to-finish farm located in France. Piglets used not be vaccinated against PCV2. They were weaned at 4 weeks of age and kept in a continuous flow nursery and 2 fattening units. All pigs were sent to slaughter at a target bodyweight of 120 kg so an increased slaughter age was the consequence of sub-optimal growth performance and lack of homogeneity. Piglets of two batches (approximately 1200 piglets) were randomly allocated to two experimental groups equally balanced according to batch, sex and weight. At weaning, one group was injected with CIRCOVAC® (Merial), 0.5 mL, IM and a *M hyo* vaccine, 2.0 mL, IM in two separated *loci* with a double-barrel syringe while the other group only received the *M hyo* vaccine. Group-based bodyweights were recorded at weaning, age and carcass weights were individually recorded at slaughter. Lung scores (Madec's grid) were recorded in approximately 180 pigs in each group according to the same sampling schedule. Statistical analysis were performed using ANOVA, Wilcoxon test, F-test or Fisher's exact test depending on the parameter. Growth parameters were analyzed only in the first batch as the lightest pigs were sold before slaughter in the second batch.
Results: No adverse reaction was observed after any of the vaccinations. Mortality rate remained low i.e. 1-2.5% in post-weaning and 3-5 % in fattening depending on the batch. Pigs vaccinated with CIRCOVAC were slaughtered 2.7 days earlier ($p<0.01$) and growth was significantly improved by 19g/day ($p<0.01$). It yielded also a significant reduction of pigs sent to slaughter older than 182 days ($p<0.01$), thus confirming the significantly better homogeneity ($p<0.01$). Lung lesion scores were significantly improved in CIRCOVAC-vaccinated pigs with: a significant increase of non-damaged lung proportion ($p<0.01$), a definite reduction of the median lung score ($p<0.01$) and a dramatic reduction of severe pneumonia ($p<0.01$), indicating a better control of *M hyo*.
Conclusion: CIRCOVAC vaccination in addition to the *M hyo* vaccination helped in the control of subclinical PCVD and EP and improved the weaning-to-slautghter growth performance of the pigs

Disclosure of Interest: B. Delhaye: None Declared, B. Boivent Conflict with: MERIAL S.A.S., G. Perreul Conflict with: MERIAL S.A.S., J.-B. Hérin Conflict with: MERIAL S.A.S., O. Merdy Conflict with: MERIAL S.A.S., F. Joisel Conflict with: MERIAL S.A.S.

Keywords: Enzootic pneumonia, PCV2, Vaccination

Viral and Viral Diseases
PCV2

PO-PT2-288

An Update On The U.S. National Porcine Circovirus Type 2 Prevalence: Before And After Wide Scale Vaccination

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Introduction: Porcine circovirus 2 (PCV2) is associated with Porcine Circovirus Associated Diseases (PCVAD), such as a syndrome causing weight loss and ill thrift. PCVAD threatened the swine industry in Europe and later North America for about 15 years. In North America PCV2 vaccines were introduced in 2006. Since then four vaccines have come into widespread use. However, there has been little research to determine whether wild virus is still circulating in the national herd, whether U.S. swine producers still observe clinical signs suggestive of PCVAD or whether the relative prevalence of the two main genotypes of PCV2(PCV2a and PCV2b) has changed in the intervening years.
Materials and Methods: Questionnaire data and blood samples were collected as part of the NAHMS Swine 2006 and 2012 studies. In 2006, blood samples were collected from up to 35 grower/finisher market pigs per farms on 185 farms in 16 states. A Capsid based ELISA was used to test for the presence of PCV2 antibodies and a TaqMan (TM) quantitative PCR was used to test whether PCV2 DNA was present in the sera of these pigs. A SYBR Green (SG) dye-binding PCR combined with melting point analysis was used to detect PCV2 genotype in sera.
In 2012, blood samples were collected from up to 17 grower/finisher market pigs per farm on 137 farms in 10 states. Capsid and Replicase based ELISAs were used to test for the presence of PCV2 antibodies to vaccine and wild type virus, respectively. SG quantitative PCR alone was used with melting point analysis to detect DNA presence and genotype.

Results: Per the questionnaire data, nationally, 31.3% and 13.7% of sites with grower/finisher pigs reported PCVAD in 2006 and 2012, respectively. The farm level prevalence for exposure (PCV2 antibodies) across years ranged from 98.9 to 100%. The Capsid ELISA from 2006 and the Replicase ELISA used in 2012 assessed whether pigs had been exposed to wild type PCV2 virus. Both tests revealed approximately 78% of animals had been exposed to virus. Nearly 83% of animals and 100% of farms sampled were infected (per PCR) with PCV2 in 2006. In 2012, less than 18% of animals and 47.9% of farms were infected. There has been a shift away from infections with PCV2a and mixed infections towards PCV2b infections between study years.
Conclusion: Prior to the use of vaccines, more farms with weaned pigs reported clinical signs of PCVAD. However, the disease remains as does exposure to wild virus. The reason for the apparent reduction in animal level infection in 2012 is likely due to vaccination and subsequent viral clearance. The shift in genotype profile may reflect a greater ability for PCV2b to out compete PCV2a.

Disclosure of Interest: None Declared

Keywords: PCV2

Viral and Viral Diseases
PCV2

PO-PT2-289

Benefit of piglet PCV2 vaccination in a herd with subclinical PCV2 infection

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Introduction: Based on serological studies, it is assumed that PCV2 is ubiquitous in the pig industry across the world. The purpose of this study was to evaluate the benefit of PCV2 piglet vaccination on ADG and mortality rate from weaning to slaughter in a herd subclinically infected with PCV2.
Materials and Methods: The study was conducted in a farrow to finish farm located in Brittany, France, positive for PRRSv, PCV2, APP, *Mycoplasma hyopneumoniae*, *and Lawsonia intracellularis*. The mortality rate from weaning to slaughter was below 5%. The ADG from weaning to slaughter puts the farm in the upper third among Brittany swine farms. Before the start of the study pigs of 120 and 140 days of age (doa) were tested positive for PCV2 by PCR. In total 929 piglets from 4 farrowing batches were included in the study. At 20 doa, one day before weaning, piglets were individually weighted, ear tagged and randomly allocated to either Group V (Vaccinated Group, N=465) or Group C (Control group, N=464). Piglets from Group V were injected with 1 ml Ingelvac CircoFLEX® by the intra-muscular route. Piglets from Group C did not receive any vaccine. The farm staff was blinded to treatment. To determine the course of PCV2 infection ten randomly selected pigs per group and per batch were designated as sample animals. In total 80 sample pigs were serially bled at inclusion, end nursery, mid and end finishing. Samples were assayed by PCV2 quantitative PCR. The primary parameters of this study were ADG and mortality rate from weaning to slaughter. Individual carcass data were collected at slaughter. Live weight at slaughter was calculated back from the carcass weight and was used together with the individual slaughter age to calculate the wean-to-slaughter average daily gain (ADG w-s). Data were analyzed using the statistical software Mintitab® (version 17). Data from the groups were compared using a t-test.
Results: PCV2 viremia was confirmed by PCR on serum samples in animals of treatment group C of all 4 batches. The level and duration of viremia varied from batch to batch. In total, 17 pigs died during the study, 9 in the vaccinated group and 8 in the control group. ADG from weaning to slaughter was significantly greater in Group V than in Group C, 719,11 vs 708,05g respectivly ($p<0.05$).
Conclusion: This study demonstrates the benefit of PCV2 piglet vaccination on ADG in a farm having already good technical results and in which PCV2 infection was not suspected to impair the performances. In the present study it was not able to determine FCR but it could be speculated that it can also be improved by vaccination as Correge *et al.* showed.

Disclosure of Interest: None Declared

Keywords: PCV2, Subclinical, Vaccination

Poster Abstracts

Viral and Viral Diseases
PCV2

PO-PT2-301

Case Report of Handling PDNS in Fattening Pigs by PCV2 Vaccination in Breeder and Piglet

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Introduction: Porcine Dermatitis and Nephropathy Syndrome (PDNS) is a clinical sign of Porcine Circovirus Associated Disease (PCVAD). PDNS is characterized by purple skin lesions with black center, fever, and lethargy, mostly affecting fattening pigs between 12-16 wks old. Vaccination is one of the tools to handle PCV2 and PDNS. This case report illustrates the real situation in pig farm in Thailand that suffered from PDNS for years and how the farm changed vaccination program in breeders and piglets and how serology and morbidity improved from 2014 to 2015.
Materials and Methods: A farrow to finisher farm with 1,200 sows, one-site and continuous flow in each unit was observed. There were gilt acclimatization, boar (AI), gestation, lactation, nursery (3-11 wks old), and fattening (12-24 wks old-600 pigs/house) units. During 2014 to Jun 2015, PCV2 was positive by serology (ELISA), serum PCR (viremia at 12-16 wks old) and tissue PCR of sick pigs. The clinical signs of PDNS occurred from nursery (8-9 wks old). Incidence was increased from 10% in nursery to 40-50% in fattening at 12-16 wks old with viremia found in this group in Dec 2014. Mortality was lower than 20% but times to market were increased. From 2014 to Jun 2015, subunit PCV-2 vaccine was applied to sow at 10-12 wks of gestation, piglets at 3 and 7 wks old, gilts at 20 wks old. No PCV2 vaccine used in boars. From Jun 2015 to Nov 2015, new PCV2 vaccine type and program was applied. The inactivated PCV2 vaccine was used 2ml in gestating sows at 8 wks of gestation, gilt at 20 wks old, and boar at every six months. Piglets were vaccinated at 5 wks old, 0.5 ml. During transition period, 9-14 wks gestating sows were vaccinated with 2 ml vaccine and 8-11 wks old pigs were vaccinated with 0.5 ml vaccine. Serum of breeders and fattening were collected and tested by ELISA (Biochek) in Dec 2014 and Aug 2015, before and after new vaccine program. PDNS morbidity rate in fattening was observed in Nov 2015.
Results: Mean S/P ratio of PCV2 in breeder were 1.797, 1.801, 1.710, 1.671 in gilts, Sow P1, P2-5, and P>5 in Dec 2014.Three months after new PCV2 vaccination, mean S/P ratio were increased to 2.072, 2.474, 2.316, and 2.256, respectively, in Aug 2015. In nursery to fattening, mean S/P ratio were 1.669, 1.361, 0.604, 0.895 in 7-8 wks old, 10-12 wks old, 14-16 wks old, and 18-20 wks old in Dec 2015 and 2.260, 1.550, 1.072, and 1.060, respectively, in Aug 2015. No PCV2 seroconversion was detected in Aug 2015. PDNS morbidity in Nov 2015 was 3/600 (0.5%) at 14-16 wks old.
Conclusion: The inactivated PCV2 vaccine in boar and earlier vaccination program in gestating sows (8 wks of gestation) can improve PCV2 immunity in breeders, piglets, and fattening pigs and reduce PDNS in fattening pigs according to the practical use in PDNS high incident farm.

Disclosure of Interest: None Declared

Keywords: PCV2, PDNS, Vaccination

Viral and Viral Diseases
PCV2

PO-PT2-028

Continuous evolution of porcine circovirus type 2 in Korea

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Introduction: Porcine circovirus type 2 (PCV2) has circular, single-stranded DNA (ssDNA) genome, which consists of two major open reading frames (ORF1 and 2). PCV2 isolates belong to three genotypes (PCV2a, PCV2b and PCV2c), based on the difference of ORF2 sequence. In 2009, molecular epidemiology study on Chinese PCV2 isolates identified the presence of novel PCV2d genotypes based on phylogenetic analysis. In particular, PCV2d genotype has drawn much attention from the public because novel PCV2 has emerging in the cases of suspected vaccine failure. Global molecular analysis suggested that genotype shift to PCV2d is currently ongoing process among pig population. The objectives of this study were to develop our knowledge on the genetic diversity of Korean PCV2 isolates and to further investigate the frequently emergence of PCV2d genotype.
Materials and Methods: Since 2009, clinical samples were collected from commercial pig farms. Total DNA was extracted from clinical samples. Extracted DNA was mixed with PCV2-specific primer set in PCR premix. ORF2 sequence or full-length PCV2 sequence was amplified with specific primers. The target band was purified and sequenced. Phylogenetic analysis were performed by neighbor-joining method with 1000 of bootstrapping values.
Results: Of total 78 samples, 58 samples were positive for PCV2 (75% of positive rate). Eighteen PCV2 isolates from 15 pig farms were sequenced. Phylogenetic analysis of ORF2 showed that five isolates were classified as PCV2a, seven as PCV2b and six as PCV2d genotype, repectively. On the basis of chronological order, 3/4/0 isolates in 2009 and 1/3/3 in 2012 and 1/0/3 in 2015 belonged to PCV2a/PCV2b/PCV2d, respectively. Notably, of five PCV2a, one isolate was classified within PCV2a-2C, which had been not detected worldwide since the last identification in 2006 in Europe. Our result indicated that PCV2d genotype was frequently detected in Korean pig farms. Interestingly, we found two cases of genotype shift (PCV2a to PCV2d and PCV2b to PCV2d) and one concurrent infection of two genotype (PCV2a and PCV2d) on the farm level, which implies continuous genotype shift to PCV2d in Korean pig farms.
Conclusion: Our study showed considerable genetic variation of PCV2 in Korea. Interestingly, continuous evolution of PCV2 directed ongoing genotype shift to PCV2d after the emergence in around 2012. Therefore, continuous surveillance of PCV2 and update on PCV2 vaccine should be needed to prepare effective strategies against emergence of novel strains from viral evolution.

Disclosure of Interest: None Declared

Keywords: None

Viral and Viral Diseases
PCV2

PO-PT2-284

Case report: changes in mortality after an alteration in the PCV2 vaccination protocol

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Introduction: PCVAD is considered to be an economically important disease because of high mortality and morbidity. Ninety seven percent of Korean swine farms vaccinated against PCV2 in 2013. However various PCV2 vaccines show differences in efficacy and safety. The objective of this study was to determine whether the change of a PCV2 vaccine can have an effect on mortality.
Materials and Methods: The field observation was conducted on a farrow to finish farm with 100 sows. This farm was repopulated in February 2013 and therefore had a good health status and was PRRS negative. Piglets are weaned at 24 days of age, and transferred to the finisher barn around 70 days of age. This farm started to use Circumvent PCV according to label with a first shot at 21 days of age and a second shot at 42 days of age in October 2013. However, several sudden deaths were observed immediately after vaccination. Therefore, it was decided in March 2014 to change the PCV2 vaccine and to use Ingelvac CircoFLEX® given according to label at 21 days of age. To evaluate the possible effect of the different vaccines on mortality, the overall mortality per batch and the animals that perished within 48 hours after vaccination were recorded. For Circumvent PCV2, 17 batches of in total 555 piglets and for Ingelvac CircoFLEX® 26 batches of in total 1071 piglets were evaluated. During the time of this observation, there were no other changes in management. Fisher's exact test was used to test differences of mortality between two vaccination groups.
Results: For the pigs that were vaccinated with Circumvent PCV, the mortality for the 48 hours post vaccination period was 7.2 % compared to 0.8 % for the pigs that were vaccinated with Ingelvac CircoFLEX® (p=0.0001). The pre-weaning mortality of two groups was 13.2% (Circumvent PCV) and 8.2% (Ingelvac CircoFLEX® p=0.0021). This resulted in 8.46 pigs weaned per sow for the pigs that were vaccinated with Circumvent PCV compared to 9.36 pigs weaned per sow in the pigs vaccinated with Ingelvac CircoFLEX®. The wean to slaughter mortality was 2.07 % (Circumvent PCV) and 1.42 % (Ingelvac CircoFLEX®).
Conclusion: Circumvent PCV vaccinated pigs had a higher pre-weaning mortality and a lower number of weaned pig per sow compared to the piglets that were vaccinated with Ingelvac CircoFLEX®. This difference was mainly observed within 48 hours after vaccination. No post mortems or other diagnostics have been applied so it is hard to speculate on the cause of the observed difference in mortality. But the fact that the mortality was chronologically associated with vaccination suggests that vaccine safety and its implication on animal's performance should be well considered when making vaccine choices.

Disclosure of Interest: None Declared

Keywords: PCV2 vaccine, safety, sudden death

Viral and Viral Diseases
PCV2

PO-PT2-100

Emergence of mummifications, abortions and stillborn piglets in a gilt herd shortly after complete repopulation

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Introduction: In November 2014 a sow farm in north Germany was repopulated with 440 gilts. In the Danish breeding facility, the pigs were vaccinated only against *Haemophilus parasuis*. Four groups of 110 gilts each were introduced into the German farm over a 4 month time period. As reported from the referring veterinarian, all animals were vaccinated against Porcine Parvovirus (PPV), Porcine Circovirus type 2 (PCV2), Influenza A virus, *Erysipelothrix rhusiopathiae* and *Mycoplasma hyopneumoniae* at the German facility. 3 ½ month after artificial insemination, gilts showed prolonged gestational periods. Increased abortion rates with big variations in the size of the piglets including mummies were observed as well as stillborn and weak piglets in different litters. In total only 65% farrowed at all. Based on these observations, PPV was suspected to be the underlying cause of disease.
Materials and Methods: Blood samples from affected sows were taken. Necropsy, histology, immunohistochemistry as well as polymerase chain reaction (PCR) was performed on affected piglets. Samples were tested for porcine reproductive and respiratory syndrome virus (PRRSV), PCV2, Parvovirus, Enterovirus, Leptospira sp. and Chlamydia. Feed was tested for Mycotoxins and for a lack of Amino acids (Arg).
Results: Diagnostic tests revealed an infection with PCV2. Circovirus DNA was detected in blood samples of the affected sows. High virus levels were evident in piglet tissue samples (heart, liver, kidney, lymph node). Characteristic histological lesions in the cardiac muscle were found in both stillborn and weak born piglets.
Conclusion: The presented case reports an unusual manifestation of a PCV2 infection of gilts regarding the clinical presentation as well as regarding the previously implemented vaccination. The cause for this unexpected reproductive disease outbreak has been identified to be a delayed vaccination with Ingelvac CircoFLEX®, which for the first three batches of gilts took place too late after introduction. As a consequence, PCV2 was able to spread among the immunologically naïve gilts during two month after placement leading to a fatal clinical disease. The fourth group, which was vaccinated immediately after arrival, was unaffected. To reduce further virus spread, re-mass vaccination was performed successfully in the herd. After implementing a strict vaccination scheme, clinical signs dissolved and reproductive herd performance improved to normal.
Conclusively, a detailed anamnesis regarding vaccination schemes is crucial for thorough diagnostic proceedings and expedient treatment and disease prevention.

Disclosure of Interest: None Declared

Keywords: abortion, PCV2, porcine

Poster Abstracts

Viral and Viral Diseases
PCV2

PO-PT2-035

Fava bean feed reduces sensitivity of PCV2-PCR on oral fluids

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Introduction: Oral fluid sampling via cotton ropes is a faster, less invasive and cheaper way to sample a large number of pigs compared to traditional blood sampling. However, the advantages of the method can only be fully appreciated, if the diagnostic validity is un-affected. In general, PCR tests are very sensitive but also very prone to inhibition by several substances. The objective of the study was to clarify, if oral fluid from fava bean-fed pigs contains inhibitory substances that affect a PCV2-PCR test.
Materials and Methods: Blood samples and oral fluid were collected simultaneously from finishing pigs in the same pen. In each pen containing up to 32 pigs, four randomly selected pigs were bled, and oral fluid was collected by leaving a chewable cotton rope hanging in the pen for approximately 30 minutes. Serum from the four blood samples were pooled prior to PCV2-PCR-analysis yielding one result from the serum and one result from the oral fluid. Almost halfway through the study period, the additional protein source for the liquid feed was changed from soybeans only to primarily fava beans.
The quantitative real-time PCR analysis for PCV2 (detection limit: 10^3 PCV2 copies per ml serum) was performed at DTU Vet in Denmark. Initially, this test was used for serum and oral fluids (old method). Later, an additional dilution procedure for the DNA extracted from oral fluid prior to PCR analysis was implemented to resolve potential inhibition of the PCR (new method).
Results: A total of 69 and 191 pen-wise pairs of serum and oral fluid were collected in the soybean and fava bean period, respectively. For serum, 83% in the soybean period and 94% in the fava bean period were positive for PCV2 virus (p=0.008). For oral fluid samples analysed by the old method, 100% in the soybean period and 65% in the fava bean period were positive for PCV2 virus (p<0.0001). Furthermore for the soybean period, a significant correlation (p<0.0001) was measured between all serum and oral fluid quantitative results with an estimated Spearman´s correlation coefficient of 0.5. For the fava bean period, this correlation was not significant.
For clarification, 10 soybean and 10 fava bean samples were analysed simultaneously by the old and the new method. Regardless of method, all soybean samples were positive for PCV2. However for the fava bean samples, only 4/10 samples were positive by the old method, whereas all samples were positive by the new method (p=0.015).
Conclusion: An increased risk of false negatives seems to exist, if oral fluid from fava bean-fed pigs is used for diagnosing PCV2 infection. Dilution of extracted DNA prior to PCR testing may solve this problem.

Disclosure of Interest: G. Blach Nielsen Conflict with: PhD Student at MSD Animal Health, J. Haugegaard Conflict with: MSD Animal Health, C. K. Hjulsager: None Declared, J. P. Nielsen: None Declared, H. Houe: None Declared

Keywords: fava beans, PCR sensitivity, PCV2

Viral and Viral Diseases
PCV2

PO-PT2-093

Investigation on the influence of sow vaccination with Ingelvac CircoFLEX® on reproductive performance in a large production system in Poland

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Introduction: Sow reproductive performance may be impaired by PCV2 infection. Clinical reproductive disease is described as a rare event; however, vaccination against PCV2 has been shown to improve reproductive performance in clinically affected and unsuspicious sow herds. This study was conducted to investigate the impact of sow vaccination on reproductive performance of a herd where no PCV2 associated clinical signs were reported.
Materials and Methods: The study was conducted at a farrow-to-wean farm with 3860 sows. Piglets were weaned off-site with approximately four weeks of age. Routine vaccination of replacement gilts against PCV2 was already implemented at two weeks after weaning and at 26 weeks of age before the trial started. Sows were grouped in weekly farrowing batches with a mean size of 130 sows. Data was recorded for twelve consecutive batches (6 groups / treatment); alternately three sow batches were not vaccinated and three sow batches were vaccinated with 2 replicates. Sows of vaccinated groups received 1ml of Ingelvac CircoFLEX® intramuscularly on the day after weaning (pre-breeding). The number of live born and weaned piglets per litter were compared between treatment groups in the subsequent farrowing. A general linear model was used to calculate differences in live born and weaned piglets per litter between treatments including the treatment group as main factor and batch size and replicate as covariates. P-values equal or below 0.05 were considered significant.
The aim of the study was to investigate, if Ingelvac CircoFLEX® vaccination before mating could improve reproduction parameters of sows in their subsequent farrowing.
Results: Batches contained 108 to 141 sows farrowing from September to November 2014. Vaccination of sows led to a numerical improvement of the number of live born piglets per litter with a mean number of 15.35 (standard deviation (SD): 0.274) piglets in litters of vaccinated sows vs. 14.72 (SD: 0.854) piglets in litters of non-vaccinated sows (p = 0.146). The mean number of weaned piglets per litter differed significantly (p = 0.047) with 11.57 (SD: 0.476) piglets in litters of vaccinated vs. 10.95 (SD: 0.351) piglets in litters of non-vaccinated sows. No effect of batch size and replicate were measurable for the analysed parameters (p > 0.05).
Conclusion: The data indicates that also in sow farms with no overt reproductive problems, PCV2 may have a negative impact on reproductive performance and sow vaccination can therefore improve performance.

Disclosure of Interest: A. Formanowski Conflict with: Boehringer Ingelheim, P. Cybulski Conflict with: Poldanor S.A., M. Adam Conflict with: Boehringer Ingelheim, R. Langhoff Conflict with: Boehringer Ingelheim

Keywords: PCV-RD, reproductive performance, sow vaccination

Viral and Viral Diseases
PCV2

PO-PT2-129

Serological and virological response after pig vaccination with Porcilis PCV M Hyo or a mixed combination vaccine against PCV2 and M hyopneumoniae

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Introduction: Porcilis®PCV M Hyo was the first RTU PCV2/Mhyo vaccine in EU. In order to choose the most effective PCV-Mhyo combination vaccine, a comparative trial was conducted in a Dutch closed pig farm with PCVAD and Mhyo problems. Pigs were sampled at multiple time points to compare PCV2 viral load as well as M hyo and PCV2serological response. Both of these measures are important to understand the infection dynamics in vaccinated- and infected herds
Materials and Methods: Trial farm: 170 sows in a 2 weeks batch production system. Three (3) week old piglets from 6 batches were randomly allocated to 3 groups and were vaccinated: P-Porcilis PCV M Hyo (n=266), X-mixed combo (n=262), C-saline controls (n=268). Pigs from different groups were commingled throughout the study.
In batch 1, 3 and 5, about 10 pigs/treatment group were blood sampled at 3, 10, 18 and 22 weeks of age. Samples were tested for Mhyo (Idexx Elisa) and PCV2 (ORF2 Alphalisa, MSD-AH; $\log_2$ titer). PCV2 viremia was tested with qPCR determining % viremic pigs and average viral load (10LogDNA copies/µl). PCV2 positive pigs were also categorized as shortly or persistently (>1 positive of 4 samples) viremic.
Results: Throughout the trial, significantly less than P (43%) or X (69%) than C (100%) pigs were PCV2 qPCR positive. In addition, significantly fewer P and X than C pigs were persistently infected, as well as significantly less P than X pigs (C=90%, X=34%, P=10%). Viremia was significantly reduced in vaccinated pigs compared to controls but also between treatments groups P and X in favor of P.
Pigs were vaccinated in the face of moderately high PCV2 maternal antibodies (C=4.7, X=4.9, P=5.6). Both X and P pigs had highest PCV2 titer by 7 wks following vaccination, while C pigs seroconverted between 10 and 18 wks of age. Overall, P pigs tended to have higher titers than X pigs at each sampling point.
At vaccination, 30% (C), 32% (X) and 17% (P) pigs were Mhyo seropositive. By 10 wks of age, none of C or X pigs were seropositive compared to 31% P pigs. At 18 and 22 wks of age, 38% of P pigs were seropositive while only 10% or less of C or X pigs were positive.
Conclusion: The results demonstrate a field infection with PCV2 and Mhyo during the trial. Vaccination significantly reduced PCV2 viremia incidence, persistently viremic pigs and viral load, and this reduction was higher in Porcilis PCV M Hyo than mixed combo pigs. In addition, Porcilis PCV M Hyo induced a better humoral immune response against PCV2 and Mhyo compared to the mixed combo vaccine. These results suggest that there are differences across PCV2 and Mhyo combination vaccines that may affect overall vaccine efficacy.

Disclosure of Interest: None Declared

Keywords: Mhyo, PCV2, viremia

Viral and Viral Diseases
PCV2

PO-PT2-202

DIARRHEA IN LATE FINISHERS CAUSED BY PCV2 INFECTION - A CASE REPORT

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Introduction: This report describes the differential diagnose of acute diarrhoea with increased mortality in a case of late finishing pigs. In the last 3-4 batches pigs showed bloody mucoid diarrhea late in finishing after the first pigs had been marked for slaughter, resulting in a preliminary diagnosis of *porcine haemorrhagic enteropathy* (PHE). After acute onset of clinical signs, affected pigs usually died within 24 hours, despite systemic treatment with Tylosin or Tiamulin.
Materials and Methods: The study farm is a finishing farm with a total of 4.500 places distributed over 3 sites. Piglets were vaccinated against *Mycoplasma hyopneumoniae* and porcine intestinal adenomatosis on the farm of origin. The live Ileitis vaccine was administered one to three weeks after placement into the nursery, applied orally via drinking bowls. Six fecal samples were taken and investigated by PCR for *Lawsonia* (*L.*) *intracellularis* and *Brachyspira hyodysenteriae*. Post-mortem examination (PME) was performed on two affected pigs and formalin-fixed samples of the small intestines were investigated histopathologically, immunhistologically (*L. intracellularis*) and by *in situ* hybridization (PCV2).
Results: Bloody content was present in small and large intestine. Macroscopically gastric ulcers were not found. PCR for *Brachyspira* spp. was negative, PCR for *L. intracellularis* was positive on 3/6 fecal samples. Histopathology revealed a moderate, diffuse, lympho-histiocytic and plasmacytic enteritis with eosinophilic granulocytes. Immunohistochemistry failed to detect *L. intracellularis*. In contrast, PCV2 was found either in cells of mucosa-associated immune follicles or in infiltrating histiocytic cells of the lamina propria. Therefore, PCV2 was suspected to be the cause of the enteric disorder. PCV2 vaccination at weaning was implemented as routine measure, mixed with a single dose of Mycoplasma vaccine using the same adjuvant. The previous clinical signs disappeared immediately once the first vaccinated pigs reached late finishing. The performance results of four non-vaccinated batches during the clinical outbreak compared to four vaccinated batches are summarized in Table 1.
Conclusion: Clinical signs and detection of *L. intracellularis* antigen in feces are not sufficient to confirm PHE in late finishing. Histopathological investigation is an essential step towards a reliable diagnosis. Even in the absence of typical clinical signs suggestive of PCVD (wasting, respiratory symptoms, PDNS) PCV2 infection has to be considered as differential diagnosis. This case report demonstrates that PCV2 infection can cause severe haemorrhagic enteritis in late finishing associated with increased mortality.

Disclosure of Interest: None Declared

Keywords: acute diarrhoea, late finishing pigs, PCV2

Poster Abstracts

Viral and Viral Diseases
PCV2

PO-PT2-126

The Importance of Monitoring PCV2 virus and PCV2 Antibodies

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Introduction: PCV2 virus (PCV2) causes economic damage and is a risk factor in swine production. Vaccination coverage is >85%. Scientific literature reports differences in protection. Subclinical PCVAD (PCVAD-SI) can occur in vaccinated herds. In protection against PCV2 infection both Virus Neutralizing antibodies (VN) and Cell Mediated Immunity (CMI) are important. Maternally Derived Antibodies (MDA) are protective and the titer height varies among piglets. High MDA titers at vaccination will interfere with vaccination and seroconversion. Seroconversion after vaccination is a clear sign of successful vaccination. When protection is optimized the resulting financial gains can be substantial, for example by creating uniformity in MDA titers through sow vaccination, combined with a better timing of piglet vaccination. Protection after vaccination can be monitored by checking for PCV2 antibodies (BioChek PCV2 ELISA) and for PCV2 viral load (BioChek PCV2 qPCR).
Materials and Methods: Literature was screened for mode of action of PCV2, PCV2 induced immunity, the importance of sub-clinical infections, differences in control of PCV2 infection by vaccination and differences in economic performance. Investigations by BioChek on PCV2 monitoring by ELISA and qPCR was included.
Results: PCV2 virus alters cytokine production, impairing the immune response. Studies have shown that vaccines can be efficacious and that vaccinated groups perform better than control groups. Control groups have more animals with PCV2 viremia and infected animals have higher PCV2 viral load. PCV2 immunity depends on VN antibodies and CMI. When sero-conversion is observed after vaccination, CMI is also induced. Groups with higher sero-conversion measured by ELISA and with a higher level of uniformity in titers showed a lower level of PCV2 (qPCR) viremia and recorded a better economic performance. When vaccination is monitored for sero-conversion and viral load, the optimal vaccination moment can be determined. The BioChek PCV2 monitoring system reports both antibody titers and viral load, and includes the Coefficient of Variation (CV%). CV% indicates the level of uniformity within a batch. Lack of uniformity in serological protection is a factor leading to the biological variation often observed in PCVAD.
Conclusion: PCVAD-SI is of economic importance. PCVAD-SI can be detected by generating information on PCV2 antibody titers and PCV2 viral load. BioChek PCV2 ELISA and qPCR test kits provide information on efficacy of vaccination, serological uniformity and quality of PCV2 control. Substantial financial gains are reported by using this system.

Disclosure of Interest: None Declared

Keywords: BioChek, Diagnostics, PCV2

Viral and Viral Diseases
PCV2

PO-PT2-244

Evaluation of the efficacy of a combination of CircoFLEX and MycoFLEX in a farm

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Introduction: PCVD (Porcine Circovirus type 2 (PCV2) diseases) and EP (enzootic pneumonia) are two of the economically important swine diseases in China. Single or co-infections with these pathogens are associated with several clinical signs, such as postweaning multisystemic wasting syndrome (PMWS), stunting, cough, diahrea, dermatitis and nephropathy. The purpose of this study was to evaluate the efficacy of the combination of Ingelvac CircoFLEX® and Ingelvac MycoFLEX® in a farm.
Materials and Methods: This study was conducted in a commercial farrow-to-finish farm with a herd size of 1000 sows in Jiangxi province in China. This farm had been vaccinated with a local PCV2 vaccine (a Cap protein subunit vaccine based on baculovirus vector; two doses at 14 and 35-days of age) and an imported *Mycoplasma hyopneumoniae* vaccine (two doses at 7 and 21-days of age), with slaughter pigs sold from September 2013 to August 2014. Beginning September 2014 through August 2015, pigs were sold that were vaccinated with the combination of Ingelvac MycoFLEX® and Ingelvac CircoFLEX®, 1 dose of 2.0ml (1.0mL Ingelvac CircoFLEX® and 1.0ml of Ingelvac MycoFLEX®, at 14-days of age) to immprove performance, convenience and compliance.
Performance records were collecte for the before and after periods.The following parameters were recorded per batch: weaning weight at 25-day age, market weight, mortality in both nursery and finishing pigs, Feed Conversion Ratio (FCR), Average Daily Weight Gain (ADWG) and drug cost per finisher.
Results: The use of the combination showed good efficacy in reducing mortality, FCR, drug cost and improving ADWG. Compared with the data of before use, the mortality of after use was reduced from an average of 11.2% to 3.9%, and FCR was reduced from an average of 2.82 to 2.51. The ADWG was improved an average of from 652.3g/d to 705.7g/d, and the drug cost also reduced by an average of 13.4 yuan (2.2 USD).
Conclusion: In this farm, the freshly mixed combination of Ingelvac MycoFLEX® and Ingelvac CircoFLEX® reduced the mortality rate, FCR and drug cost, and improved ADWG by 53.4g/d, which illustrated the superior efficacy of the combination. Additionally, the combination made vaccination easier for both people and pigs by reducing stress with a single-shot injection of the two different vaccines.

Disclosure of Interest: None Declared

Keywords: None

Viral and Viral Diseases
PCV2

PO-PT2-186

Observations of a licensed trivalent PRDC vaccine for PCV2, M.hyo and PRRS

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Introduction: Porcine Respiratory Disease Complex (PRDC) is a multi-pathogen disease that can cost owners >$10/pig. Immune protection against clinical disease associated with these pathogens is one control method. A trivalent PCV2, M.hyo and PRRS vaccine, 3FLEX® (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, USA), has been available in the U.S. marketplace since 2010 and used to vaccinate hundreds of thousands of pigs. The purpose of this field study was to evaluate pigs vaccinated with 3FLEX® compared to a non-vaccinated group following challenge with PCV2 and PRRS.
Materials and Methods: Study pigs (n=675) were weaned from two typical commercial sow units that were demonstrated PRRS stable (herd category II-A) on weaned pig PRRS PCR (HMC, Ames, USA) testing. To prevent shedding of vaccine from the vaccinated group to the control group, pigs receiving 3FLEX®(2ml, n=387) were housed approximately 1.0 km north/downwind from non-vaccinated control (NVC) group (n=288) for 48 days. No PRRS virus (serum PRRS PCR) was detected in NVC pigs 12 hours prior to transport to the challenge site. Following the vaccination period, pigs were transported by treatment group, and assigned to pens based on pig size (small, medium, large) and balanced by treatment. One pen per barn held pigs from NVC and 3FLEX® groups to be challenged with a proven oral PRRS (type 2, 4000TCID50) and PCV2 (8 logs) positive tissue homogenate (5 gallon feed, top-dressed with 500 mL of tissue homogenate/pen) one week post-placement. An additional intramuscular challenge with PRRS virus (2mL, 2 logs) was made 10 days following the oral exposure. A subset of pigs were euthanized and necropsied to confirm a valid challenge by observing lesion development at five weeks post challenge.
Results: The following differences in performance variables were observed in 3FLEX® vaccinated vs. NVC, respectively: Initial body weight (BW) 27.9kg vs. 28.0kg (P=0.81); Final BW(kg) 118.9 vs. 112.7 (P<0.0001); ADG(grams) 875 vs.816 (P<0.0001); Mortality(%) 5.1 vs. 28.2 (P<0.0001); Culls(%) 11.8 vs. 27.5 (P<0.0001).
Conclusion: The results of this trial suggest that 3FLEX® provides protection to pigs challenged with PRRS and PCV2 as compared unvaccinated cohorts. To the authors' knowledge, this is the first large scale field study to successfully demonstrate individual pig PRRS and PCV2 challenge following 3FLEX® vaccination. In the face of a PRRS and PCV2 challenge, use of a trivalent vaccine mixture is an option for managing the economic losses associated with PRDC and the single injection reduces time and labor required for vaccination against these costly diseases compared to multiple, single-antigen vaccine or two dose protocols.

Disclosure of Interest: None Declared

Keywords: 3FLEX, Challenge, Trivalent

Viral and Viral Diseases
PCV2

PO-PT2-252

The effects of different vaccination dosing and timing protocols on porcine circovirus type 2 viremia and post vaccination serologic responses

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Introduction: *Mycoplasma hyopneumoniae (Mhyo)* and PCV2 cause significant economic losses to the U.S. swine industry. Circumvent® PCV-M G2 (Merck AH, USA) protects against both pathogens using two dosing options: single, 2mL intramuscular (IM) dose at 3 weeks of age (WOA) or older, or 1 mL IM as early as 3 days of age followed by 1mL dose 3 weeks later. The objective was to evaluate different vaccination dosing and timing protocols on PCV2 viremia and post vaccination serologic responses.
Materials and Methods: This study was conducted in four separate flows in a large production system. One flow was a parity 0 to 1 sow farm that provided sows for the three other sow farms. Pigs (n=521) were randomly assigned by weight within litter to treatment groups just prior to weaning at 21-28 days of age: A) 2mL, 3 WOA; B) 1mL 3&7 WOA; C) 1mL 3&9 WOA. In the finisher, pigs were evenly dispersed throughout the barn by treatment and mixed with non-study pigs. Pigs were weighed at allocation, 9 WOA, and prior to marketing at 24 WOA. Blood samples were collected at allocation and 7, 9, 16, and 24 WOA. Serum was tested for PCV2 viremia by PCR in pools of five at ISU Veterinary Diagnostic Lab. Environmental challenge was assessed by monitoring oral fluids for PCV2 by PCR at each blood sampling. Serum was tested for PCV2 antibodies by IFA, reported as the reciprocal of the geometric mean (GM) titer and *Mhyo* antibodies by ELISA performed at 3, 7, and 9 WOA.
Results: No pigs were viremic at 3, 7, 9, 16, and 24 WOA. Oral fluid rope samples at 7, 9, 16, and 24 WOA were PCV2 PCR low positive. Group B seroconverted to *Mhyo* at a high rate in the presence of *Mhyo* maternal antibody at 3 WOA. PCV2 maternal antibody at 3 WOA reduced IFA titers at 9 WOA. Group B pigs with titers $\leq$640 at 3 WOA had a GM of 3,044 at 9 WOA while pigs with GM$\geq$1,280 had 1,428. Average daily gain (ADG) for A, B, and C was 0.827, 0.842, and 0.834 pounds in nursery and 1.991, 2.012, and 2.029 pounds in finisher, respectively and was not significantly different. Mortality rates were A 4.6%, B 5.7%, and C 5.2% and did not differ significantly.
Conclusion: No viremia was detected in any pigs, possibly due to the low challenge level as assessed by oral fluid samples. The reason for the low challenge level is unknown. Previous testing of these flows indicated that active PCV2 infection was occurring. However, potential variability in endogenous challenge level is a limitation in field studies. Serologic testing shows that B pigs are able to mount an immune response to both *Mhyo* and PCV2 in the presence of maternal antibody. Additional handling for a second vaccination did not negatively impact ADG.

Disclosure of Interest: None Declared

Keywords: IFA, PCR, PCV2

Poster Abstracts

Viral and Viral Diseases
PCV2

PO-PT2-099

Onset of Immunity of Porcilis® PCV ID concurrently administered with Porcilis® M Hyo ID Once

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Introduction: The objective of the described studies was to assess the onset of immunity of a recently registered intradermal (ID) vaccine, Porcilis® PCV ID, given alone or concurrently with Porcilis® M Hyo ID Once. The studies were done in Dutch pig herds.
Materials and Methods: Pigs were vaccinated ID using the needle free IDAL injector with Porcilis® PCV ID-PCV, with Porcilis® PCV ID and Porcilis® M Hyo ID Once concurrently-PM, with Porcilis® M Hyo ID Once-M or left untreated.
PCV2: 15, 19-23 day old pigs per group were vaccinated with PCV, PM or left untreated. 2 weeks later pigs were infected with a field isolate of PCV2 and 18 days later all pigs were necropsied. Serum samples were examined for PCV2 antibodies (Ab) and the presence of PCV2 nucleic acid by quantitative PCR. Faecal swabs and at necropsy, inguinal lymph node, tonsil and lung were collected for detection of PCV2 nucleic acid.
M hyo: 20, 18-24 day old pigs per group were vaccinated with M, PM or left untreated. 3 weeks later pigs were infected with a virulent *M. hyo* strain and lung lesions were scored upon necropsy, 3 weeks post-challenge, according to Goodwin & Whittlestone. Blood samples for serology were collected.
Results: PCV2: At vaccination, all groups had similar Ab titers. After vaccination and challenge, the mean Ab titer in PCV and PM pigs was significantly higher than the mean Ab titer of the controls. Sera and swabs were negative for PCV2 nucleic acid at challenge. During the challenge period the viral load for PCV and PM pigs was significantly reduced compared to the controls. The viral load in lymphoid tissues and organs was also significantly lower in PCV and PM pigs compared with controls. There was no significant difference between PCV or PM pigs.

M hyo: At vaccination, all pigs were serologically negative for *M. hyo,* and positive for PCV2. PM pigs remained at the same PCV2 antibody level until challenge, whereas antibody levels decreased in the controls. The lung lesion scores of M and PM pigs were significantly lower than those for the controls (M=3.6; PM=3.3; C=17.7). There was no significant difference between M and PM pigs.
Conclusion: Based on the results, concurrent use of Porcilis® PCV ID and Porcilis® M Hyo ID Once protects against PCV2 and *M. hyo* from two and three weeks post-vaccination on, respectively. In addition, the intradermal vaccination has several benefits over intramuscular vaccination, including no needle breakage or carcass damage, lower vaccine volume and more operator/animal friendly.

Disclosure of Interest: None Declared

Keywords: intradermal vaccination, onset of immunity, PCV2

Viral and Viral Diseases
PCV2

PO-PT2-127

Field efficacy of Porcilis® PCV ID concurrently administered with Porcilis® M Hyo ID Once

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Introduction: PCV2 is involved in Porcine Circovirus Diseases and *M. hyopneumoniae* is the primary agent of enzootic pneumonia. Vaccination against both minimizes the economic impact resulting from infection. The objective of this study was to assess efficacy of an intradermally administered vaccine, Porcilis® PCV ID, given alone or concurrently with Porcilis® M Hyo ID Once. The study was carried out in a Hungarian pig herd with confirmed PCV2 and M.hyo infections.
Materials and Methods: A total of 1322 healthy, 18-24 day old suckling piglets were allocated randomly, within litters, to one of four treatment groups: 1) PCV-vaccinated intradermally with Porcilis® PCV ID, 2) PM-vaccinated intradermally with Porcilis® PCV ID and Porcilis® M Hyo ID Once concurrently, 3) M-vaccinated intradermally with Porcilis® M Hyo ID Once, 4) Control-untreated. The primary efficacy parameters were PCV2 viraemia, M. hyo lung lesions at the slaughterhouse and average daily weight gain (ADWG) during finishing. Secondary parameters were the overall ADWG (from vaccination to slaughter), mortality, morbidity, PCV2 faecal shedding, and pleurisy lesions.
Results: The PCV pigs had a significantly better weight gain than the Control group and group M during finishing and overall. The differences with the Control group were 48.7 and 30.8 g/day for the two study periods, respectively and the differences compared to M group, were 37.3 and 24.7 g/day. The PM pigs had a significantly better weight gain than the Control group and group M during all phases of the study. The differences with the Control group were 19.4, 58.3 and 41.9 g/day for the three study periods, respectively. The differences compared to M group, were 23.0, 46.9 and 35.7 g/day, respectively. Vaccination with Porcilis® M Hyo ID Once significantly reduced the mean lung lesion score (Goodwin and Whittlestone) from 7.7 and 6.4 in respectively the control and PCV group to 3.4 and 4.2 in respectively the PM and M group. PM and PCV pigs were significantly less viraemic than the Control and M pigs. Pleurisy scores, morbidity and mortality were not statistically different across treatments. PCV2 faecal shedding was significantly lower in PCV and PM pigs compared to the Control and M pigs.
Conclusion: Based on the study results, intradermal vaccination with Porcilis® PCV ID given as a single dose, alone or concurrently with Porcilis® M Hyo ID Once is efficacious under field conditions. In addition, intradermal vaccination with a needle free IDAL injector has several benefits over intramuscular vaccination, including, ease of application, reduced volume, no muscle damage due to needle breakage, less stress on animals and administrator.

Disclosure of Interest: None Declared

Keywords: field efficacy, intradermal vaccination, PCV2

Viral and Viral Diseases
PCV2

PO-PT2-275

A comparative field efficacy study comparing two multinational one shot PCV2 vaccines in a 1,800 sow farm in Korea

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Introduction: PCVAD is considered to be an economically important disease. In Korea, most swine farms can control PCVAD by vaccination against PCV2. PCV2 vaccines have been developed by several global and local (Korean) animal health companies. However the different PCV2 vaccines show differences in efficacy and safety (da Silva N., 2014). Vaccine efficacy, safety and return of investment are the most important criteria for to the selection of a vaccine. The purpose of this study was to compare two PCV2 vaccines developed and marketed by two global animal health companies in terms of efficacy and cost –effectiveness.
Materials and Methods: The field observation was conducted on a three-site production farm with 1,800 sows. Piglets are weaned at 21 days of age and transferred to the nursery site. At around 70 days of age growers are transferred to the finisher site. To compare the two different vaccine programs, piglets weaned between May 2014 and Sep 2014 were included in the field study. Farm performance parameters after weaning were recorded from 7,609 pigs weaned May 2014 to July 10, 2014 (Fostera PCV, treatment group A) and from 8,608 pigs weaned July 10, 2014 to September 2014 (Ingelvac CircoFLEX® treatment group B). Animals of both treatment groups were vaccinated at 21 days of age according to label. There were no other changes except PCV2 vaccination program. To evaluate the performance of the pigs in the two different treatment groups, mortality rate, average market weight and average daily weight gain (ADG) were recorded for each group. Fisher's exact test was used to test differences of mortality between two vaccination groups.
Results: Fostera PCV vaccinated pigs (Group A) showed higher mortality than Ingelvac CircoFLEX® vaccinated group (Group B). The wean-to-slaughter mortality was 10.2% for group A and 6.2% for group B ($p<0.0001$). Average market weights of group A and B were 111.2 kg and 112.7 kg respectively. Average daily weight gain (ADG) from weaning to slaughter of the animals in group A and B were 782.4g and 808.6g respectively. The differences in performance results into a difference in margin over feed and medicine of 6.53 US$ per pigs in favor for the Ingelvac CircoFLEX® vaccinated pigs.
Conclusion: The findings of this study are in line with other studies that show that Ingelvac CircoFLEX is more efficacious compared to other PCV2 vaccines (Jung., 2011 and Kim and Seo., 2012). The economic evaluation highlights the importance of vaccine efficacy. When it comes to the selection of a PCV2 vaccine efficacy and return of investment together with vaccine safety are the most important criteria. Vaccine cost should only be considered in the context of vaccine efficacy and return of investment.

Disclosure of Interest: None Declared

Keywords: Efficacy, mortality, PCV2 vaccine

Viral and Viral Diseases
PCV2

PO-PT2-024

PCV2 vaccine cross-protection: Identification of sequences in successfully vaccinated field cases

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Introduction: It is necessary to evaluate emerging PCV2 isolates in both control and clinical cases in order to determine the importance and impact of novel mutations. The primary objective of this study was to identify PCV2 isolates in herds where production performance was meeting the systems' expectations, no clinical PCVAD signs were apparent and the organization was satisfied with vaccine.
Materials and Methods: Farms (n=48, 10 unique systems) were selected using the following criteria: 1) use of Ingelvac CircoFLEX® at weaning age, 2) pig owner and veterinarian were satisfied with vaccine, 3) pig performance was meeting systems' expectations and 4) no clinical health issues suggestive of PCVAD present. Serum (n=432 samples;23 farms), oral fluids (OF;n=168 samples;24 farms) and lung homogenate (n=1 sample) were collected. Individual pig serum and OF samples were PCV2 tested (HMC, Ames, IA) using TaqMan real-time PCR reagents with a detection limit of below 3.5 genomic equivalents/reaction. Lung tissues were sent to ISU-VDL for PCV2 PCR. Farms' (n=16) samples were targeted for lower Cq values with a PCR cycle quantity (Cq) ≤32 for serum (n=16), Cq≤30 for OF (n=11) and for tissues (n=1; Cq=19.3) to be sent to U of MN for PCV2 capsid gene sequencing with quality analysis performed within DNAstar (Madison, WI). Each isolate was further characterized based on standards and dendrograms were created.
Results: Twenty-seven farms had PCV2 PCR positives (12, 15 and 5 from serum, OF, lung homogenate, respectively) of which 44 samples (24, 17 and 3 from serum, OF, lung homogenate, respectively) were sent for sequencing. There was an overall 91% sequencing success rate (87% for serum, 94% for OF, 100% for tissue homogenate). Forty total samples were successfully sequenced and characterized as PCV2a (n=3), PCV2b (n=21) or PCV2d (n=16).
Conclusion: Vaccination strategies have decreased the presence of PCV2, yet it remains in the barn environment and vaccinated populations. Though not reflective of the population, the biased sampling of PCV2 PCR positive samples based on Cq level allowed for a more economically beneficial success rate on sequencing. No farms with multiple sequences on samples had homologies less than 100% (amino acid comparison), indicating that the same strain was found multiple times in the same farm in multiple pigs. Although there were forty sequences, via capsid sequencing there was only a maximum 3% heterology amongst these samples. In this study, vaccinated pigs showed no clinical signs of PCVAD and met the systems' performance expectations, while still being exposed to a wide-range of modern PCV2 strains. Although mutations may occur the commercial PCV2 vaccines appear to remain efficacious.

Disclosure of Interest: None Declared

Keywords: Cross-protection, Sequencing, Vaccine

Poster Abstracts

Viral and Viral Diseases
PCV2

PO-PT2-216

Effect of colostral immunity on the antigenic activity of the "VERRES-CIRCO" vaccine and distribution of IgG/IgM after challenge of pigs with PCV2

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Introduction: Vaccination against PCVD is widely practiced, two principal consequences being the possibility of colostrum-derived antibodies affecting post-vaccination immunity and the need to be careful when interpreting the results of diagnostic tests, especially if antibody levels are measured.
The present article looks at the influence of colostral immunity to PCV2 on the formation of post-vaccination immunity. Another goal was to determine the dynamics of anti-PCV2 IgG/IgM detection depending on vaccination schemes.
Materials and Methods: In the present study we used a group of clinically healthy 3-week-old piglets whose peripheral blood was free of PCV2. The study was carried out at the All-Russian Research Institute of Experimental Veterinary Medicine. On the basis of the presence or absence of IgG, the animals were divided into 4 experimental groups (n=6): IgGposVac; IgGnegVac; IgGposNVac; IgGnegNVac. The "VERRES-CIRCO" subunit vaccine against PCVD was used for immunisation. PCV2 "TM-2014" ($10^{4,0}TCID_{50}$/ml) used as a challenge had been isolated from a pig with PMWS and propagated in the PAMs. Detection of PCV2 DNA and anti-Cap PCV2 IgG/IgM in pig serum samples was performed using a commercial PCR kit and a commercial ELISA kit, respectively.
Results: Vaccination of seropositive pigs did not induce production of IgM, but led directly to an increase of the IgG concentration, which was typical for secondary immune response. Vaccination of seronegative animals induced production of IgM and then, following isotype switching, of virus-specific IgG. Similar results were obtained after experimental challenge of piglets. Production of post-challenge IgM only took place in piglets that had been seronegative initially or in piglets after the complete disappearance of colostral antibodies. Challenge of seropositive piglets only resulted in a quantitative change of IgG concentration. Despite the presence of IgG at the moment of vaccination, the level of IgG after vaccination did not alter.
Conclusion: Detection of virus-specific IgG and DNA of PCV2 using ELISA and PCR, remains the most commonly used approach for laboratory diagnosis of PCVD in Russia.
Results obtained in this study indicate that the presence of maternally-derived antibodies does not interfere with the formation of strong vaccine-induced immunity. We also conclude that it is important not only to test samples for IgG and viral DNA presence, but also to assess IgM levels to understand a given herd's PCV2 and PCVD status.

Acknowledgments
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Disclosure of Interest: None Declared

Keywords: IgG, IgM, PCV2 vaccine

Viral and Viral Diseases
PCV2

PO-PT2-098

The use of oral fluids as a tool for monitoring porcine circovirus type 2 viremia

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Introduction: Porcine circovirus type 2 (PCV2) is a ubiquitous pathogen that causes significant economic losses in growing pigs. While effective commercial vaccines are available, the recent emergence of a possibly more virulent variant, PCV2d, poses challenges for understanding the potential impact of PCV2d on subclinical disease due to viremia and overall environmental levels and shedding. Accordingly, a monitoring tool for evaluating herd-level infection status is needed. Oral fluids are routinely used as a non-invasive sample for monitoring other population diseases such as PRRS and IAV-S. The objective of our study was to determine if testing oral fluids by PCR could be used to monitor the PCV2 infection status of growing pigs rather than the more common method of testing serum for viremia by PCR.
Materials and Methods: Samples were collected in one barn on each of 32 finishing farms, representing 28 sow flows. All pigs received commercial PCV2 vaccine according to the production system's standard protocols. One week prior to the first marketing event, 6 oral fluid samples (OFL) were collected per barn and from the same pens, 5 pigs that were observed to chew the rope were bled to obtain serum. Samples were tested by PCR at the Iowa State University Veterinary Diagnostic Laboratory using standard methods. Serum samples (SER) were pooled by pen. Statistical analyses included Chi square and linear regression.
Results: A total of 960 market hogs were sampled generating 192 OF and SER. Overall, 39.1% of SER and 77.6% of OFL were positive. Comparing the two sample types on a pen basis, 21.9% were negative on both, 39.1% were SER^- and OFL^+, 0.50% were SER^+ and OFL^- and 38.5% were positive on both ($p<0.05$). Within pen correlation of CT values was highly significant. On a barn basis, 37.5% were SER all negative, 15.6% SER all positive, 6.3% OFL all negative and 62.5% OFL all positive. The relationship between the two tests was not significant on a pos/neg basis but was highly correlated when comparing the average CTs.
Conclusion: Positive PCR results on oral fluids for PCV2 have been regarded as representing the level of environmental virus with little correlation with the level of infection/viremia in the pigs. This study proposes that oral fluids represent more than environmental viral load and can be used to estimate the levels of PCV2 viremia and viral shedding within a population of growing pigs. This monitoring technique can be a valuable tool for veterinarians and producers because it is easy to perform and is non-invasive to pigs compared to other methods such as collecting blood or tissue.

Disclosure of Interest: None Declared

Keywords: Oral fluids, PCV2, Viremia

Viral and Viral Diseases
PCV2

PO-PT2-125

PCV2 load in lymphoid tissue at slaughter after vaccination with Cirbloc®

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Introduction: Porcine circovirus type 2 (PCV2) is a highly prevalent pathogen in pig farms worldwide. Reduced performances in apparently healthy pigs may be associated with subclinical infection. Prevention is possible with PCV2 vaccination. The objective of the study was to evaluate the efficacy of Cirbloc®, a vaccine against the expression of PCV2, in decreasing the virus load in lymphoid tissues of slaughtered pigs in PCV2 affected commercial farms.
Materials and Methods: Randomized, blinded and negative controlled field trials were carried out at three farrow-to-finish farms; two located in Hungary and one in France. 997 piglets (300-397 per farm) were vaccinated with one dose (2 ml i.m.) of the vaccine on the left side of the neck at 3 weeks of age. They were compared with a same number of control animals treated with 2 ml of PBS under the same conditions. The animals were randomized to be similar in age, maternal origin, body weight and sex ratio. They remained mixed in the same pens until the end of the study. At commercial slaughter at about 5 months after vaccination, inguinal and mediastinal lymph nodes were collected from at least 45 pigs per group, in each farm, to quantify PCV2 load. The viral DNA was extracted from the samples by the QIAamp DNA Mini Kit and was detected and quantified by real-time PCR using PCV2 specific primer pairs. The effect of “Farm” (Factor A) and “Treatment” (Factor B) were investigated by using two-way ANOVA assuming a fixed-factor design.
Results: Out of the 997 pigs, 289 were randomly selected (143 vaccinated and 146 control ones) and sampled at slaughter at the end of the fattening period. The virus load was significantly lower for the vaccinated group taking into account all three farms ($p<0.0001$). Although the virus levels in the lymph nodes were different among the farms ($p<0.0001$), the interaction of “Farm” and “Treatment” factors was non-significant for both mediastinal and inguinal lymph nodes ($p\geq0.05$ respectively). This means that the vaccination was consistently beneficial irrespective of the farm and type of the lymph node. The virus load reduction was 0.76–1.20 log10 in the mediastinal lymph nodes and 1.19–1.45 log10 in the inguinal lymph nodes. In other words there was 6 to 28 times reduction of PCV2 copy numbers depending on the farm and the lymph node concerned.
Conclusion: At slaughter at about 24 weeks of age, the virus load in lymph nodes of the vaccinated pigs was markedly lower and this was shown to be consistent from one farm to another. Vaccination with Cirbloc® was shown to be effective in reducing the PCV2 load in lymphoid tissues of animals sampled at commercial slaughter time.
Cirbloc® is a trade mark of Ceva Santé Animale

Disclosure of Interest: None Declared

Keywords: PCV2 vaccine, virus load

Poster Abstracts

Viral and Viral Diseases
PCV2

PO-PT2-128

Results of the vaccination against PCV2 with Cirbloc® on the growth in a farm in Hungary

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Introduction: Porcine circovirus type 2 (PCV2) is a highly prevalent pathogen in pig farms worldwide (up to 100% of pigs may be seropositive to PCV2 at slaughter). Subclinical infection is associated with reduced growth in apparently healthy pigs. Prevention against the expression of PCV2 is possible by vaccination. The objective of the study was to evaluate the efficacy of Cirbloc® vaccine on the performances of pigs expressed by their average daily weight gain (ADWG) in a commercial farm in Hungary.
Materials and Methods: A randomized, blinded and negative controlled field trial was carried out at a farrow-to-finish pig farm affected by PCV2, in Hungary. Three hundred piglets were vaccinated with one dose (2 ml) of Cirbloc® vaccine (batch No: 29B10) intramuscularly on the left side of the neck at 3 weeks of age and compared with 300 piglets treated with 2 ml of PBS under the same conditions. The animals were weighed individually at inclusion (21±3 days of age), at the end of nursery (ca. 14 weeks of age) and at the end of fattening (ca. 24 weeks of age). Before and after each weighing session the calibration of the scales was checked by using one or more standard weights. The ADWG was expressed in grams per day (g/d). The ADWG was compared between groups using a mixed-effects model including treatment group and the animal's age as fixed effects, litter of origin and pig as random effects. The individual ADWG of any animal that died, was culled or excluded before the weighing session was not included in the calculation of mean ADWG.
Results: Over the whole rearing period (from inclusion to slaughter) the growth was significantly higher of 18 g/d in the vaccinated group (686±86 g/d, mean±SD) than in the control group (668±92 g/d) - group-by-time interaction, p=0.002. During the fattening period (transfer to slaughter), the growth difference of the vaccinated group was even higher (39 g/d) with statistical significance: 834±134 g/d versus 795±134 g/d - group-by-time interaction, p<0.0001.
Conclusion: The vaccination against PCV2 with Cirbloc® helped to improve the performances expressed by the growth in a contaminated farm. The growth improvement was at the highest during the fattening period.
Cirbloc® is a Trademark of Ceva Santé Animale.

Disclosure of Interest: None Declared

Keywords: PCV2 vaccine, weight gain

Viral and Viral Diseases
PCV2

PO-PT2-219

Field safety evaluation of Cirbloc® vaccine against PCV2 in fattening pigs

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Introduction: PCV2-infection is widespread and essentially all pig herds are infected with PCV2 but relatively few express PCV2-diseases (PCVD) which include severe systemic PCV2 infection, PCV2-associated pneumonia, PCV2-associated enteritis, PCV2-associated reproductive failure, and Porcine Dermatitis and Nephropathy Syndrome (PDNS). Prevention is possible by vaccination. The objective of this trial was to evaluate the safety of Cirbloc®, vaccine against the expression of PCV2, in pigs.
Materials and Methods: A comparative, controlled, randomized and blinded field trial was performed on a commercial farrow-to-finish farm affected by PCV2, in France. 798 healthy piglets on two successive weaning batches were assigned to either a test or a control group and vaccinated at 3 weeks of age with either one dose of Cirbloc® or one dose of PBS (2 ml i.m., respectively). The safety observations were carried out on a focus group of 60 pigs (31 vaccinated and 29 control ones). They were observed for 14 days.
Species/breed: Swine: fattening pigs: Crossbred: Naïma x Pietrain
Primary parameters:
(a) immediate post vaccination reaction: within 1 hour after administration
(b) rectal temperature (D*-1, D0, D0+2h, D0+4h, D0+6h, D0+12h and daily from D1 to D4)
(c) general health (D-1, D0, D0+2h, D0+4h, D0+6h, D0+12h and daily from D1 to D14)
(d) local reactions (D0, D0+4h, D0+6h, D0+12h, daily from D1 to D14)
Secondary parameter:
Weight gain during the safety observation period: the pigs were weighed at D-1, D7 and D14.
Mortality and morbidity: for evaluating their relationship with the vaccination.
Results: No adverse event related to the vaccination was recorded during the safety period.
No mortality and no morbidity related to the vaccination with Cirbloc® was observed on the vaccinated pigs in safety group. Three piglets died in control group (attributed to enteritis by necropsy). Percentage of morbidity and mortality calculated on all included animals was not significantly different between groups.
No immediate reaction was observed.
The average individual maximum increase of rectal temperature was 1.2°C in the vaccinated group. No increase was observed in control group.
No abnormal general or local reaction was observed.
The average daily weight gain (ADWG) was not significantly different in the vaccinated group than in the control group from D0 till D14.
Conclusion: The safety of Cirbloc® under field conditions was confirmed for each of the considered parameters.
*D = Day
Cirbloc® is a trade mark of Ceva Santé Animale

Disclosure of Interest: None Declared

Keywords: PCV2 vaccine, safety

Viral and Viral Diseases
PED

PO-PW1-046

A challenge study evaluating the pathogenicity of classical PEDV variant compared to that of pandemic variant isolated in Thailand in 3 day-old pigs

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Introduction: Porcine epidemic diarrhea (PED) is a devastating enteric disease in pigs characterized by vomit, acute watery diarrhea and high mortality in young pigs. In Thailand, PED was first reported in 2007 and pandemic variant was responsible for the pandemic outbreak. Since then, PED has developed into an endemic stage in which many herds experience recurrent outbreaks. In 2014, there was a report on the emergence of classical variant PED virus (PEDV) isolated from the farm providing intramuscular vaccination on sows. However, the severity of clinical disease was milder compared to the previous outbreak caused by pandemic variant. Due to the lack of information on its pathogenicity, this study aims to determine the virulence of the classical variant PEDV compared to the pandemic variant PEDV isolated in Thailand.
Materials and Methods: Ten sows from a PED free herd were randomly selected and two piglets were chosen by 1st and 2nd suckling order from each of ten sows. At 3 days of age, piglets were weaned from sows and randomly divided into two treatment groups consisting of G1 and G2. Pigs in G1 and G2 were orally challenged with PEDV isolates EAS1 and CBR1, representing the classical and pandemic variants, respectively. Following challenged, pigs were monitored daily for clinical diarrhea with score ranging from normal to severe (1-5). To determine the fecal shedding pattern, fecal sample were collected and assayed for the presence of viral RNA using RT-PCR. At 5 days post challenge, all piglets were euthanized. Villous-to-crypt-depth ratio (VCR) of small intestine were histopatholgically examined. Data were analyzed using one-way ANOVA and least significant difference (LSD). The VCR values were displayed as mean (standard error of the mean; SEM).
Results: The clinical scores of G1 at 1 day-post-challenge (DPC), 2 DPC, 3 DPC and 4 DPC were 2.4, 2.8, 2.6 and 3.6, respectively, while the G2 were 2.8, ,2.6 ,3.4 and 4.4. The viral shedding at 1-4 DPC were detected 60, 100, 100 and 100 percent in G1, whereas, 0, 100, 100 and 100 percent in G2, respectively. The VCR of duodenum, jejunum and ileum (0.79 (0.08), 1.02 (0.07) and 0.99 (0.10), respectively) of G2 were significantly less than G1 (1.59 (0.17), 1.19 (0.08), 1.24 (0.12), respectively).
Conclusion: According to the results, the pandemic variant PEDV isolated (CBR1) in Thailand tend to be more virulent than the classical one (EAS1).

Disclosure of Interest: None Declared

Keywords: porcine epidemic diarrhea virus (PEDV), Thailand, virus

Viral and Viral Diseases
PED

PO-PW1-072

EFFECT OF TWO PORCINE EPIDEMIC DIARRHEA (PED) VACCINES ON PREWEAN MORTALITY UNDER FIELD CONDITIONS IN MEXICO.

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Introduction: The main impact of Porcine Epidemic diarrhea (PED) virus is high mortality in piglets between birth and weaning and as a consequence pig producers have serious economic losses. Since 2014, Mexico has been positive to PED virus as diagnosed by the PCR test.
Materials and Methods: The aim of this study was to assess the effect of two vaccines against PED (manufactured by Zoetis™ and Harrisvaccines™ respectively) compared to no vaccination. During a multi-site outbreak that occurred in March, 2015, the vaccines were applied in sows and gilts and unvaccinated controls were also kept. Both PED vaccines were used according to their label directions (intramuscular injection, pre-farrowing). Diagnostic testing revealed that some sites were affected by the prototype PED virus and others with the Indel strain. Response variables, measured on a per litter basis, were: total pigs born, pigs born alive, mummies, stillborn, pigs weaned pigs, dead pigs and mortality rate. The data were analysed by means of a mixed linear model including the random effects of farm and farrowing group and the fixed effects of parity (1 or greater than 1), vaccine strain (Harrisvaccines™ and Zoetis™) & challenge strain (Prototype or Indel). 31492 records were included in the data base.
Results: All vaccinated groups showing low premortality weaning and better piglet quality. Gilts (1st farrow) yielded smaller litters and had higher mortality rates (12.4 vs 13.4 and 47.5 vs 38.0% respectively, P<0.01) than sows (2nd farrow or greater). Animals in the control group had more deaths per litter than those treated with either vaccine (7.8, 3.0 and 2.8 for control, Zoetis and Harrisvaccines™, respectively. P<0.05) and higher prewean mortality rate (65.4, 24.4 and 24.9 for control, Zoetis and Harrisvaccines™, respectively. P<0.05). Differences between challenge strains were found for pigs weaned, number of deaths and mortality rate per litter (9.1 vs 6.7; 3.1 vs 5.0 and 25.8 vs 42.3 for Indel and Prototype strains), although these differences could also have been influenced by site factors.
Conclusion: The use of either PED vaccine reduced prewean mortality independently of challenge strain (Indel or Prototype viruses). PED vaccines can help to reduce negative impact of PED disease in infected herds. The use of the vaccines are an excellent tools to control the disease in addition to biosecurity measures.

Disclosure of Interest: None Declared

Keywords: PED, PED Vaccines

Poster Abstracts

Viral and Viral Diseases
PED

PO-PW1-071

EFFECT OF TWO PORCINE EPIDEMIC DIARRHEA (PED) VACCINES ON IMMUNOGLOBULINS PRODUCTION DURING FIELD PED OUTBREAK IN MEXICO.

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Introduction: The main impact of Porcine Epidemic diarrhea (PED) virus is high mortality in piglets between birth and weaning and as a consequence pig producers have serious economic losses. Since 2014, Mexico has been positive to PED virus as diagnosed by the PCR test.
Materials and Methods: The study was conducted during a PED outbreak in March 2015 and assessed the effect of two PED vaccines (manufactured by Zoetis and Harrisvaccines™) on the secretion of anti-PED IgA and IgG in comparison to a non-vaccinated control group. The vaccines were applied according to the manufacturers' directions (intramuscular injection pre-farrowing), but with some animals receiving two doses and some only one. Colostrum samples were taken aseptically from sows and gilts after farrowing. As facilities for immunoglobulin measurement are not available in Mexico samples were sent to Iowa State University (ISU) and analyses performed according to ISU procedures. The data were analysed by means of a mixed linear model including the random effects of farm and farrowing group and the fixed effects of parity (1 or greater than 1), treatment (control, Harrisvaccines and Zoetis), day after application (0, 7 and 14 days), doses (0, 1 , 2), challenge strain (Prototype or Indel) and two way interactions. Response variables were the titers of IgA and IgG. 671 records were included in the database.
Results: Parity did not affect titers of IgA (P>0.05), but there was a difference between gilts and mature sows in IgG (1.74 and 1.57 respectively, P<0.05). The vaccinated groups had higher titers of IgG (2.60, 2.95 and 2.90 for control, Harrisvaccines and Zoetis respectively) and IgA (0.96, 1.51 and 2.09) (P<0.05). Titers of IgG (2.60, 2.73 and 3.07 for 0, 7 and 14 days) and IgA (0.96, 1.68 and 1.78) increased with time after vaccination (P<0.05). Doses affected the amount of IgG (2.60, 2.88 and 3.15 for 0, 1 and 2 doses) and IgA (0.96, 1.80 and 1.51) (P<0.05). Differences were found among challenge strains for IgG (2.85 and 2.89 for Indel and Prototype, respectively) and IgA (1.33 vs 1.75) (P<0.05). No interactions were detected.
Conclusion: Both vaccines stimulated IgA & IgG independently of challenge strain. Parity influences immunoglobulin levels with gilts producing less than sows, consistent with the belief that gilts are less efficient at immunoglobulin production.

Disclosure of Interest: None Declared

Keywords: IgG, IgA, PED, PED Vaccines

Viral and Viral Diseases
PED

PO-PCO1-009

Elimination of porcine epidemic diarrhea virus (PEDV) and porcine delta coronavirus (PDCoV) and validation of a herd infection status classification

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Introduction: PEDV and PDCoV entered the US in 2013 infecting 56% of the sow herds. The PIC multiplication system totaling 92,000 sows in North America had accumulated a record of minimal impact from PRRS or *M. hyopneumoniae* in past years; but from November 2013 to April 2014, 47% of the breeding herds became infected with PEDV and/or PDCoV. Likely because of their remote location and biosecurity, none of the genetic nucleus or sire line nucleus farms were affected. This abstract summarizes the elimination process in 17 sow farms and the validation of the herd infection status classification system proposed by NPB/AASV.
Materials and Methods: Once a diagnosis was definite, controlled oral exposure of the breeding herd (including replacement gilts) with the resident live virus began. No pigs entered the herd until viral shedding had ceased based on objective assessments. Piglets were weaned off-site and sanitation was intensified during the project. Fostering between litters was limited to the first 24 hours or ceased altogether. Piglet processing and handling protocols were altered to minimize viral transfer between litters. To monitor transmission in the positive unstable (category I) herds, fecal swabs were collected from 30 different litters and tested by PCR every second week. When three consecutive negative tests were accumulated, the herd was classified as transitionally negative (category II) and sentinel pigs could be introduced. The lack of clinical signs or evidence of infection in the sentinel pigs roaming the alley-ways for five weeks allowed the herd to be classified as provisionally negative (category III). Negative serology test in 30 sentinels or bi-weekly weaned pig PCR testing for six months allowed the herd to recover negative status (category IV).
Results: By December 2014, 100% (17/17) of the elimination programs were successfully completed. It took 20 (7–28) weeks for the herds infected with PEDV and 15 (12–17) weeks for the herds infected with PDCoV to consistently wean PCR-negative pigs. No virus was detected after the introduction of sentinel pigs in category II herds suggesting that PCR testing of pigs due to be weaned is an appropriate indicator for transmission. More than a year later, no new or "reactivated" infections have been detected and 100% of the grow-finish flows have verified negative status and have been introduced to customer populations without incident.
Conclusion: The protocol based on whole-herd exposure, intensive sanitation and one-directional pig movement was able to consistently eliminate PEDV and PDCoV from breeding herds. NPB/AASV proposed classification was a useful and accurate tool to coordinate logistics and communication during the elimination efforts.

Disclosure of Interest: J. P. Cano Conflict with: PIC, T. Riek Conflict with: PIC, R. Thompson Conflict with: PIC, T. Snider Conflict with: PIC, J. Geiger Conflict with: PIC, J. W. Lyons Conflict with: PIC

Keywords: Elimination, PDCoV, PEDv

Viral and Viral Diseases
PED

PO-PCO1-011

Efficacy evaluation of regular vaccination in a PED-positive farm with a new high titer PEDv inactivated vaccine (newPED-X)

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Introduction: A new strain of Porcine Epidemic Diarrhea virus (PEDv) occurred in the Republic of Korea in November 2013 and continues to impact the swine industry up to now. The new strain was sequenced and the results showed that the spike protein gene of this newly prevalent PEDv is 99.64%~99.81% homologous to the PEDv isolated in USA from 2013 to 2014.
CAVAC acquired ISU46065IA13 strain (genotype 2a), a field isolate by Iowa State University, USA, and developed a new high titer PEDv inactivated vaccine (newPED-X). Upon development of the vaccine, CAVAC applied this vaccine regularly to a farm suffering from PEDv and monitored the changes in the farm's status.
Materials and Methods: The farm has 2,300 sows and operates a one-site rearing system. PED first occurred in this farm in February 2014 and as treatment, feedback program was implemented. However, the condition of weaned piglets deteriorated due to persistent diarrhea of suckling piglets in the farrowing house and the weight decline of weaning piglets. In order to normalize the farm, they started to vaccinate sows with newPED-X twice before farrowing.
Results: 1. Virus shedding of PEDv in relation to vaccination of newPED-X.
PEDv was detected in the feces of suckling piglets after feedback. However, PEDv was not detected in the feces in the farrowing and piglet houses at 4 months and 10 months after vaccination with newPED-X.
2. Growth rate following weaning after newPED-X vaccination
The number of weaned piglets after feedback did not differ significantly but the condition of the weanlings deteriorated due to the persistent diarrhea caused by PEDv in the farrowing houses. However, the condition of weaned piglets improved and the growth rate following weaning increased by 2.1% after vaccination with newPED-X. This resulted to an increase of 1,312 market hogs per year and is equivalent to approximately at least 0.4 M USD. Moreover, R.O.I. after newPED-X administration was calculated at 9.33.
Conclusion: It was demonstrated that regular vaccination of newPED-X on sows had a positive effect on reducing economic losses due to death and diarrhea of suckling piglets. It was confirmed that usage of the vaccine is not only beneficial for the herd's health but also provides a profit of at least 9.33 times more than the vaccination cost.
The vaccination of sows can decrease the diarrhea and death in sucking piglets, but farms must still have additional quarantine measures against PEDv circulation and continuously apply newPED-X to sows since the maternal derived antibodies remains until 6 weeks old only.

Disclosure of Interest: None Declared

Keywords: PEDV, vaccine

Viral and Viral Diseases
PED

PO-PW1-069

Study on trial vaccine using porcine epidemic diarrhea virus recently isolated in Korea

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Introduction: Porcine epidemic diarrhea caused by PEDV infection inflicts severe damages to swine industry. Though several attenuated strains such as CV777 and SM98 were used to vaccine production, recently field isolated strains were different from vaccine strains genetically as well as serologically. Recent PEDV isolates in Asia and USA show more contagious and more severe mortality than previous PEDV. Continuous vaccine strain development is essential for effective PEDV prevention and control. Therefore new field strain isolation and cultivation in the laboratory is first step for effective vaccine development against new variant. This study shows pathogenicity and immunogenicity of PED-CUP-B2014 strain currently isolated in Korea.
Materials and Methods: Clinical samples for PEDV isolation are prepared from feces and intestinal homogenates of piglets that was show typical PEDV infected symptoms. Spike protein specific real-time RT-PCR was tested for identification of PEDV positive sample. RT-PCR positive samples were mixed with sterilized PBS and centrifuged at 4,200*g* for 10 minutes at 4°C. The supernatants were filtered through a 0.22-μm filter and used as inoculums for virus isolation. Virus isolation and culture was performed in Vero cell line. Phylogenetic analysis was performed using the nucleotide sequences of spike protein of the six PEDV viruses from this study as well as other PEDV strains. Lab scale trial vaccine was prepared by several different concentration of PED virus and used with same adjuvant and some additive. Post vaccination pig serum neutralization antibody titer was measured by standard method.
Results: Virus isolation was attempted with PEDV-PCR positive feces and PEDV-PCR positive intestine homogenates was inoculated on Vero cells. The inoculated Vero cell was showed typical PEDV CPE such as cell fusion and syncytium formation. The cell observed CPE was cultured continuously in order to isolate PEDV. PEDV isolate was efficiently propagated and maintained in Vero cell cultures. After more than 60 continuous passages, isolated PEDV titer was reach to $10^{8.0}TCID_{50}$/ml. The nucleotide sequence of spike of the PEDV isolate was different from that of previous isolated PEDV. SN titer of each vaccine group was depending on concentration. Highest SN titer was 160 in the group vaccinated with $10^{7.0}$ $TCID_{50}$.
Conclusion: In this study, new variant PEDV in Korea was isolated and also successfully cultured on the Vero cell. Phylogenetic analyses show new PED-CUP-B2014 completely different from previous PEDV. Trial Vaccine of the PED-CUP-B2014 raised enough SN antibody titer to prevent PEDV infection and diarrhea.

Disclosure of Interest: None Declared

Keywords: PEDv vaccine candidate, SN titer

Poster Abstracts

Viral and Viral Diseases
PED

PO-PW1-053

Multiplex RT-PCR for inclusive detection of major diarrheal viruses in pigs with multiple infections

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Introduction: Viral diarrhea severely damage pig industry, causing tremendous economic loss worldwide, especially during the porcine epidemic diarrhea virus (PEDV) outbreaks in recent years. The most common viruses causing diarrhea are PEDV, transmissible gastroenteritis virus (TGEV), and porcine rotavirus A (PoRV-A). In the past years, some new viruses like porcine kobuvirus (PKV) and porcine sapovirus (PoSaV) were discovered from pig intestinal contents. It is very difficult to make differential diagnosis on porcine viral diarrhea due to complicated pathogens in the gastrointestinal track and high similarity in clinical signs and pathological changes presented when diarrhea occurs. In this report, we developed a multiplex RT-PCR for rapid detection and differential diagnosis on viral diarrhea caused by the above mentioned viruses.
Materials and Methods: Specific primer sets targeted to TGEV and PEDV N genes, PoRV-A gene, and PKV and PoSaV polyproteins were designed based on highly conserved regions after extensive sequence analysis on target genes. Viral RNAs were extracted and subjected to reverse transcription using hexamer random primers. PCR amplification was performed with individual primer sets and different combination of all primers using total cDNA as a template. Amplified segments were in different sizes for each virus and vary from 200 bp to 1000 bp. Primer concentrations and annealing temperature were optimized to obtain a better amplification. Lately, limit of detection and specificity of this multiplex RT-PCR were carried out for validation purpose. Finally, around 400 clinical fecal samples were tested with this multiplex RT-PCR.
Results: The results showed that the primer sets we designed functionally worked for all these five diarrheal viruses under the same amplification conditions. This multiplex RT-PCR could detect as less as 10 target cDNA copies and have no cross reactivity with other porcine viruses like porcine reproductive and respiratory syndrome virus, foot and mouth disease virus, classical swine fever virus, pseudorabies virus, and reovirus. Field samples analysis using this multiplex RT-PCR revealed that viral diarrhea in pig herd in China was mainly caused by PEDV, with some extent of combination with other diarrhea viruses.
Conclusion: These results suggest that the multiplex RT-PCR is useful for rapid and accurate identification of five major pathogenic viruses in pigs with multiple infections.

Disclosure of Interest: None Declared

Keywords: Diarrheal viruses, Multiplex RT-PCR

Viral and Viral Diseases
PED

PO-PW1-065

Evolution and protein changes in Porcine Epidemic Diarrhea Virus from the United States

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Introduction: Due to the introduction of porcine epidemic diarrhea virus (PEDV) into North and South America, PEDV has caused severe economic losses for global swine producers. Historically, shorter viral genes, including the envelope, membrane, nucleocapsid, or the S1 portion of the spike gene were sequenced to understand the evolution of PEDV. In this study, we sequenced the whole genome of 93 US PEDV strains to understand the origin and phylogenetic relationships compared to global PEDV strains.
Materials and Methods: Between January and December 2014, a total of 93 whole genome sequences were generated and combined with the 126 PEDV whole genome sequences from GenBank, which exclude vaccine strains. Nucleotide and amino acid entropy analyses were performed. A concatenated genome was constructed for each sequence, and recombination and Bayesian analysis was performed. In addition, the putative pAPN receptor-binding residues were analysized and modeled to compare differences between American and global PEDV strains.
Results: Entropy analysis of the nucleotide and amino acid sequences revealed more variablity in the NSP2 and NSP3 regions, as well as the S1 domain. Evidence of recombination was found in NSP2, NSP3, NSP14 through NSP16, the S1 domain, and the nucleocapsid gene. To effectively conduct Bayesian analysis, recombinant regions were removed from the genome. A time to most recent common ancestor (tMRCA) of September 2010 - August 2012, and July 2009 – July 2011 was calculated for the major and minor clades of PEDV sequences, respectively. An evolutionary rate of 6.2×10^{-4} substitutions/site/year was estimated for the global PEDV strains, which was similar to the US PEDV sequences (5.5×10^{-3} substitutions/site/year). The estimated substitution rate for the S1 gene was 1.5×10^{-3} substitutions/site/year. Modeling of the pAPN receptor binding domain (RBD) revealed that Asian PEDV strains contained more amino acid differences in key binding areas than US PEDV strains.
Conclusion: Higher entropy levels in the NSP2 and NSP3 regions, as well as the S1 domain, suggest a higher selection pressure on those regions. While the sequence data from Asia and Europe is limited, our analysis points to an Asian origin for the American PEDV introduction, and multiple PEDV lineages were introduced into the US. Similarities in the pAPN regions between the DR13 vaccine strain and US sequences could suggest some efficacy of vaccine strains to protect against US PEDV. However, a higher evolutionary rate in the S1 domain makes developing a long-term vaccine problematic. Continued research into the dynamics of key regions within the PEDV genome may reveal novel interventions to protect against PEDV infections.

Disclosure of Interest: None Declared

Keywords: bayesian analysis, protein modeling

Viral and Viral Diseases
PED

PO-PW1-064

Effect of direct fed microbial Bacillus subtilis C-3102 on enteric health in nursery pigs after challenge with Porcine Epidemic Diarrhea Virus (PEDv)

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Introduction: This study examined the effects of feeding *Bacillus subtilis* C-3102 (Calsporin® Calpis Co. Ltd., Japan) at the target inclusion rates of 0 CFU/g, 500 000 CFU/g and 1 000 000 CFU/g on intestinal health in weaned pigs after challenge with Porcine Epidemic Diarrhea virus (PEDv).
Materials and Methods: A two by three factorial design composed of three diets containing 0 CFU/g or 500 000 CFU/g or 1 000 000 CFU/g of Calsporin® and PEDv or sham challenge was conducted. Pigs were 14 days old at the start of the study and PEDv naïve. Ten pigs were randomly allocated to each treatment group and were housed in groups of five. Pigs were fed the treatment diets for 23 days. On day 19, pigs were challenged with PEDv-positive or -negative cell culture by gavage and necropsied at four days post inoculation (dpi). Five small intestine segments were collected per pig using a standardized technique. Histopathology slides were prepared for measurement of villus to crypt height ratios (VCR) and atrophic enteritis (AE) scoring for each section. PEDv immunohistochemistry (IHC) was performed on all slides and semi-quantitatively scored based on the percentage of enterocytes showing a positive signal. Atrophic enteritis scores were based on the presence and severity of enteritis. All scoring was completed by a single blinded veterinary pathologist. Average daily gain (ADG) and mortality was compared across treatment groups at four dpi. Responses were analyzed using linear mixed models. Comparisons among groups were assessed using F-tests followed by Tukey's t-tests for multiple comparisons. Differences were considered significant at the level of $P < 0.05$. All analysis was performed on SAS® 9.4.
Results: There were significant differences in IHC, AE and VCR between PEDv positive and PEDv negative pigs fed 0 CFU/g Calsporin®. Within PEDv positive groups, there were significant reductions in IHC and AE between pigs fed 0 CFU/g Calsporin® and treatment groups that did receive Calsporin®. VCR was greater in PEDv positive pigs fed Calsporin compared to pigs on the 0 CFU/g diet. There were no significant differences in average daily gain and mortality between any groups.
Conclusion: The findings from this study support an association between administration of Calsporin® and improved IHC scores, histopathology and villus to crypt ratios in nursery pigs challenged with PEDv compared to cohorts that did not receive Calsporin®. The impact of these parameters on morbidity, mortality, ADG and feed efficiency during the entire fattening period is unknown and should be assessed with an additional study of longer duration and larger sample size.

Disclosure of Interest: None Declared

Keywords: direct fed microbial, PEDv

Viral and Viral Diseases
PED

PO-PW1-068

Assessment of intestinal barrier function over a time course of porcine epidemic diarrhea virus (PEDV) challenge in growing pigs

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Introduction: In pigs, PEDV causes reduced growth performance, villus atrophy, and impairment of intestinal barrier function and integrity. The objective of this study was to determine the extent to which a PEDV challenge alters jejunum morphology, epithelial apoptosis, crypt cell proliferation, and tight junction proteins over a 14-day infection.
Materials and Methods: Thirty-two mixed-sex Choice Genetics maternal line approximately 4 weeks-of-age (BW=9.49±1.38 kg) and naïve for PEDV were allotted into 2 treatments: sham (Controls) and PEDV inoculated. Barrows and gilts were distributed equally among 8 pens per treatment and allowed free access to a corn-soybean meal based diet and water. The 16 PEDV pigs were intragastrically inoculated with 10^3 $TCID_{50}$/ml of PEDV isolate on day 0. On 2, 5, 7, and 14 days post-inoculation (dpi), 4 pigs per treatment were euthanized for sample collection. Formalin-fixed samples were paraffin-embedded, sectioned, and stained routinely with H&E as well as immunohistochemically for PEDV antigen, tight junction proteins (claudin 4 and claudin 2), and cellular proliferation (Ki-67). Villus height, crypt depth and villus:crypt ratios were compared for each group. DNA fragmentation (i.e., apoptosis) was assessed by TUNEL assay on sections of fixed jejunum. Caspase 3 and 7 activity of frozen samples was also used to assess apoptosis using a commercial kit. Treatment, dpi, and treatment by dpi interactions were determined with the individual pig as the experimental unit.
Results: There was no statistical difference in PEDV IHC score ($P = 0.361$) among infected pigs between 2 and 5 dpi and PEDV antigen was not detected after 5 dpi. Claudin 2 IHC score was greater ($P < 0.05$) in the villus tip of Controls than PEDV pigs; however, there was no difference in claudin 2 along the sides of villi. Claudin 4 staining was numerically lower in PEDV pigs at 2 and 5 dpi, but this was not statistically significant. The PEDV challenge also resulted in time-dependent changes in villus height and crypt depth ($P < 0.05$). PEDV pigs had more ($P < 0.001$) Ki67 positive nuclei detected than Controls. There was an interaction ($P < 0.05$) between treatment and dpi for caspase 3 and 7 activity; Controls had greater activity at 5 dpi, but PEDV pigs had greater activity at 7 dpi. No difference between treatments was observed at 2 and 14 dpi.
Conclusion: In summary, PEDV infected pigs had greater cellular proliferation, time dependent variability in apoptosis signaling, reduced expression of the barrier protein claudin 2, and decreased villus height compared with Controls. This suggests a time dependent impairment of intestinal barrier function through at least 5 dpi in PEDV infected pigs.

Disclosure of Interest: None Declared

Keywords: intestinal function, pigs, porcine epidemic diarrhea virus (PEDV)

Poster Abstracts

Viral and Viral Diseases
PED

PO-PW1-033

THE PERSISTENCE OF THE PORCINE EPIDEMIC DIARRHEA VIRUS ON INFECTED FINISHING PIG FARM DURING TEN MONTHS PERIOD

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Introduction: Porcine epidemic diarrhea virus (PEDV), a member of the family *Coronaviridae*, is an enveloped virus with a single-stranded, positive-sense RNA and causes porcine epidemic diarrhea, characterized by acute watery diarrhea, dehydration, vomiting and high mortality in nursery pigs. The objective of the present study was (i) to evaluate the duration of the virus persistence and (ii) to identify if the same virus strain was circulated on the infected finishing farm during ten months period.
Materials and Methods: PEDV infection was identified on a large finishing pig farm with about 8.000 fatteners for the first time in January 2015. A total 51 pools of fecal samples were collected from this farm after the first detection of virus to monitor the virus persistence. Sampling was performed between January 2015 and October 2015 on the weeks 1, 3, 4, 14, 23, 24, 25, 27, 32, 35, 36, 38 and 40. After RNA extraction, PEDV nucleic acids was detected by commercial real-time RT-PCR (virotype® PEDV/TGEV, Qiagen). For direct Sanger sequencing 11 PEDV positive samples were amplified also by pan-coronavirus RT-PCR to amplify 440 nt long fragment of highly conserved RNA-dependent RNA-polymerase gene.
Results: During 10 months period the observed clinical picture on the infected farm was watery diarrhea usually recognized in first two weeks after each introduction of new pigs on a farm. Out of 51 tested samples 29 (56,8 %) were detected positive by real-time RT-PCR in weeks: 1, 3, 4, 14, 23, 35, 36, 38 and 40. The obtained cycle threshold values (Ct) for positive samples were between 12.55 and 31.31 (average Ct = 22.7), confirming detection of PEDV from high to low viral load in a feces. The phylogenetic comparison of 390 nucleotides of the RNA polymerase gene of 11 positive samples showed between 99.7 % and 100 % nucleotide identity. This confirmed the circulation of the same PEDV strain during 10 months on a farm. All detected sequences belong to low virulent INDEL PEDV, closely related to strain GER/L00719/2014 detected in Germany.
Conclusion: This work has demonstrated that the same strain of PEDV can persist on infected finishing farm for at least 10 months if the biosecurity measures are not implemented. The fecal shedding on the farm is the main source of the infection for each new group of introduced pigs. Persistently infected farms can be a reservoir of PEDV in infected area and real threat for breeding herds.

Disclosure of Interest: None Declared

Keywords: persistence, pig, porcine epidemic diarrhea virus

Viral and Viral Diseases
PED

PO-PW1-055

VALIDATION OF THE ID SCREEN® PEDV INDIRECT ELISA

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Introduction: Porcine epidemic diarrhea (PED), caused by the PED virus (PEDV), is an infectious and highly contagious viral disease of pigs characterized by severe diarrhea, vomiting and dehydration. Diagnostic methods to confirm PEDV infection are virus isolation, the direct fluorescent antibody (FA) test or PCR. Antibodies may be detected either by the immunoperoxidase assay (IMPA), immunofluorescence assay (IFA), viral neutralisation test (VNT) or ELISA. The ELISA method offers the advantage of being cost-effective and easy to implement for high throughput testing. This study presents validation data obtained with the ID Screen PEDV Indirect ELISA.
Materials and Methods: The ID Screen PEDV Indirect ELISA may be used with individual porcine serum or plasma (domestic swine or wild boar samples). The kit includes microplates coated with recombinant PEDV nucleoprotein antigen and an anti-multi-species horseradish conjugante.
Due to limited access to well-defined positive samples, sensitivity was evaluated with sera from natural or experimental infections, with US or EU strains. Field samples were from farms where the presence of PEDV had been confirmed by RT-PCR. All samples were tested by IFAT. Some samples were available in very limited volumes and could not be tested on all tests.
Results: Diagnostic specificity for the iELISA, evaluated on 512 sera from areas where the virus has not been reported in recent years, was 99.2% ($IC_{95\%}$ 98.0 ; 99.7).
Out of 15 IFAT-positive samples, 12 were found positive by the iELISA and one was just above the cut-off value. Two samples giving doubtul results by IFAT were clearly positive by the iELISA.
Conclusion: This indirect ELISA may be be used to peform vaccination follow up and to determine PEDV antibody levels in sows prior to farrowing, to confirm exposure to PEDV in control programs, or to confirm freedom from PEDV infection.

Disclosure of Interest: None Declared

Keywords: Diagnostic test, ELISA, PED

Viral and Viral Diseases
PED

PO-PW1-048

Survivability of Porcine Epidemic Diarrhea Virus in Slurry

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Introduction: Introduction
Porcine Epidemic Diarrhea (PED) virus is a major enteric swine disease of economic significant in many Asia country and PED was first recognized in late April of 2013 in USA[1].
According to some research, PED virus survived in manure slurry for 28 days at 4℃ and possible longer[2].
The main purpose of this study is to investigate the time to inactivate of the PED virus in manure slurry on farm.
Materials and Methods: *Experimental farm* : This farm has 4barns with 1600 pigs as finishing site. PED has occurred on January 23, 2014, and all pigs were removed after 7 days from this farm.
PED virus detection : which have been posted depopulation on the day 41 to 161, to verify the presence of PED virus. The samples were tested via PED virus PCR.
Inoculation in piglets : The samples which post depopulation, day82 and day107, were passed into ped virus naïve to 5days old piglets via oral-gastric tube. These pigs served as a bioassay to detect the presence of infectious PED virus. Fecal swabs were collected every 24 hours. Swabs were tested via PED virus PCR. Necropsy & IHC (Immunohistochemistry) for small intestine was performed on 48 hours and 72 hours.
Results: PED virus PCR test of slurry was positive until 161days post depopulation.
Inoculated piglet via slurry at 82 days(a) post depopulation was infected with PED virus. But inoculated piglet via slurry at 107 days(b) post depopulation was not infected with PED virus.
PED virus can present in slurry during 161 days post depopulation. But It doesn`t mean infectious virus.
And PED virus in slurry has possibility to infect pigs during 82 days since post depopulation.

Conclusion: These results suggest that PED virus in slurry could be longer has infectivity than we know. Therefore, management strategy of slurry is necessary to control PED virus in the farm.

Disclosure of Interest: None Declared

Keywords: None

Viral and Viral Diseases
PED

PO-PW1-077

The Growth Kinetics of Porcine Epidemic Diarrhea Virus strain Pingtung 52

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Introduction: Porcine epidemic diarrhea (PED) is a highly contagious disease caused by porcine epidemic diarrhea virus (PEDV) infection, characterized by watery diarrhea, vomiting, dehydration, and weight loss in swine (1). PEDV has an enveloped, single-stranded, positive-sense RNA genome of ≈28 kb, belongs to the order *Nidovirale*, the family *Coronaviridae*, genus *Alphacoronavirus* (1). PEDV was adapted to serial propagation in Vero cell cultures by adding trypsin to the medium (2). The growth and titers with different multiplicity of infection (MOI) of PEDV in Vero cell culture were characterized in this study.
Materials and Methods: Vero cells were grown in minimum essential medium (MEM) (Gibco) supplemented with 10% fetal bovine serum, 1% Penicillin/Streptomycin (10,000 Units and 10 mg per ml) and maintained at 37 ºC in a humidified 5% CO_2 incubator. When PEDV strain Pingtung 52 was inoculated to Vero cells, minimum essential medium supplemented with 0.3% tryptose phosphate broth, 0.02% yeast extract, and 10 µg/ml trypsin. The MOI of one-step and multi-step growth kinetics were 5 and 0.1, respectively. At 0, 4, 6, 8, 12, 24, 36, 48, and 72 hours post infection (hpi), the cell culture fluids were collected after 3 freeze-thaw cycles and tested for PEDV titers by real-time PCR.
Results: The one-step (MOI 5) and multi-step (MOI 0.1) growth kinetics of PEDV strain Pingtung 52 in Vero cells revealed that Pingtung 52 has a short replicative cycle in Vero cells (6-8 h). Both growth kinetics showed a lag phase of about 6 h during virus replication, followed by exponential growth that lasted 30 h. The viral titer of multi-step growth kinetics was lower than that of one-step growth kinetics until 24 hpi. Virus yields of both growth kinetics reached the top at 36 hpi and the multi-step growth kinetic had exceeded by 0.5 log10. A typical coronavirus induced cytopathic effect appeared at 12 hpi, and near hundred percent of multinucleated cells were observed at 24 hpi.
Conclusion: The results of this study indicate that PEDV strain Pingtung 52 displayed similar growth kinetics with other PEDV isolates.

Disclosure of Interest: None Declared

Keywords: Growth curve, PEDV

Poster Abstracts

Viral and Viral Diseases
PED

PO-PW1-039

Fecal shedding and antibody response in four PEDV infected swine breeding farms

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Introduction: Porcine epidemic diarrhea virus (PEDV), a member of the genera *Alphacoronavirus* in the family *Coronaviridae*, causes an acute and highly contagious enteric disease characterized by severe enteritis, vomiting, watery diarrhea and high mortality in seronegative neonatal piglets. In the last years, PED caused significant economic losses in swine industry in Asia and United States and since 2014, PEDV has also re-emerged in Europe. Two main PEDV variants circulate worldwide but the so-called S-INDEL variant, considered a mild strain, is the prevalent among the European countries. To gain insight about pathogenicity, viral loads and antibody response, temporal patterns of shedding of the S-INDEL variant were evaluated in naturally infected piglets from four different outbreaks.
Materials and Methods: A longitudinal study on 4 breeding farms, naturally infected with the PEDV S-INDEL variant, was conducted between January and September 2015. Clinical data, fecal swabs and blood were collected from 30 newborn piglets at 15-30 days intervals during 2-4 months. A quantitative Real time PCR (qPCR), targeting the spike gene, was applied to determine viral loads. A competitive MAbs-based antibody ELISA test was used to detect antibodies to PEDV.
Results: In all the farms, diarrhea was observed in sows in gestation and in the farrowing unit. Mortality in piglets in the 4 farms was 10%, 25%, 40% and 50%, respectively. Percentage of PCR positive animals varied greatly from the beginning (58-100%) to the end (0%) of the study period. Clinical signs were present in 96% of PCR positive animals. Viral load in suckling pigs at 3-6 days from birth ranged from 9.2 to 3.5-log genome copies/gr. After 2-4 weeks post infection only few piglets still showed detectable levels of virus and clinical signs. The 46% of the sows showed anti-PEDV antibodies at delivery but only few piglets (3%) showed detectable antibody and absence of clinical signs at 3-6 days of age. Most of the piglets in the four farms developed antibody responses within 3 weeks of age and they remained stable till the end of the study (60-100 days of age).
Conclusion: Different PEDV circulation profiles were observed within the four infected farms, probably due to the different infection starting time. Quantitative PCR, clinical records and serological monitoring showed to be useful tools to understand the dynamic of PEDV infection and could be used to implement appropriate control measures.

Disclosure of Interest: None Declared

Keywords: Antibody response, Fecal Shedding, PEDV

Viral and Viral Diseases
PED

PO-PW1-056

Lactogenic immunity following oral exposure with porcine epidemic diarrhea virus

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Introduction: Porcine epidemic diarrhea (PED) is a devastating enteric disease, caused by PED virus (PEDV), a RNA virus in genus *Alphacoronavirus*, family *Coronaviridae*. At present, the disease causes severe economic losses worldwide. Following an outbreak, oral administration of all sows in the herd with minced intestine (intestinal feedback) of PEDV infected pigs are suggested and the sow herd will produce healthy pigs within 2-4 weeks post exposure. The lactogenic immunity of sows following the intestinal feedback should be better understanding. Therefore, the field study was conducted to investigate the PEDV specific lactogenic immunity of sows following oral exposure to PEDV and level of maternally derived antibody in pigs farrowing from PEDV exposed dams.
Materials and Methods: The present study was conducted in two pig farms with inventory of 2,400 sows each, herds A and B. Herd A was a PEDV free herd. Herd B experienced PED outbreak a year ago. Both herds were 10 kilometers away and matched in breed of sows and management system. In each herd, one hundred externally produced gilts at 22 weeks of age were introduced from a PEDV free herd to an isolation facility of each herd. Upon arrival, one hundred gilts in herd A were orally administered with 10 grams of minced intestinal sample of piglets infected with PEDV (10^4 $TCID_{50}$/ml) for 2 consecutive days, and at 12 weeks of gestation. Gilts in groups B were left as control. Colostrum and milk were collected at 7, 14 and 21 days post farrow (DPF) and assayed for antibody by viral neutralization (VN), and ELISA IgG and IgA specific against spike protein.
Results: PEDV exposed group had significantly higher VN in colostrum and milk than non-exposed group. Antibody level as measured by ELISA IgG and IgA demonstrated that sows in non-exposed group had PEDV specific antibody titer below the cut-off level. Sows in the exposed group had numerically higher level of IgA in colostrum than that of IgA. In milk samples, IgG continuously decreased to level below cut-off level at 7 DPF and maintained at that level until 21 DPF. In contrast, IgA maintained at level above the cut-off level and significantly higher that IgG at 7-21 DPF.
Conclusion: The results of the study demonstrated that PED specific IgG and IgA were detected in colostrum of sows following oral exposure with minced intestine of PEDV infected pigs. In milk samples, however, IgA was significantly higher than IgG. This evidence suggests that the proper immunization of gilts prior to introduction into the herd should be the key.

Disclosure of Interest: None Declared

Keywords: Porcine epidemic diarrhea virus, lactogenic immunity, oral exposure

Viral and Viral Diseases
PED

PO-PW1-058

Characterization of humoral immune responses in sera and oral fluids of weaned pigs following experimental PEDV infection/reinfection

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Introduction: Porcine epidemic diarrhea virus causes effusive diarrhea in pigs of all ages and significant mortality among pre-weaning pigs. A key to preventing PEDV spread is stringent movement control of animals and fomites contaminated with the virus combined with surveillance. Serology can be a key tool utilized in the control, understanding disease epidemiology and surveillance. Antibodies against PEDV have been detected in swine sera after infection with PEDV by IFA, ELISA, and SVN tests. However, detailed characterization of humoral immune response against PEDV challenge/re-challenge and serological correlates of protective immunity have not been described.The objective of this study was to characterize the humoral immune ontogeny in sera and oral fluids, including viral protein specificity of antibody response, in naïve weaned pigs following experimental PEDV infection/reinfection
Materials and Methods: Ninety-seven, 3-week-old pigs were allocated into control and challenged groups. Challenged pigs were orogastrically inoculated with 1 ml of10^3 PFU/ml of PEDV isolate (US/Iowa/18984/2013). The pigs were monitored for clinical signs and fecal shedding of the virus. Serum and oral fluid samples were collected on day 0 and every 7 days till dpi 76 and tested by IFA, ELISA, SVN and Western immunoblot (WIB). IFA was optimized and performed by using virus infected Vero cells and BHK-21 cells transiently expressing PEDV structural proteins. ELISA was optimized for simultaneous detection of IgG, IgM and IgA antibodies against PEDV
Results: Antibody responses to PEDV were detected as early as 7 dpi and 14 dpi in serum and oral fluid respectively. Serum IgA and IgG ELISA antibodies peaked at 21 and 28 dpi and started to decline. Among the viral structural proteins, serum antibodies response against S, and N proteins was detected as early as 7 dpi followed by M and E proteins. SN activity showed up at 14 dpi spiking after rechallenge at 56 days but overall titers were low. Oral Fluid IgA and IgG ELISA antibodies peaked at 35 and 21 dpi and started to decline at different rates. IgA was major antibody isotype in oral fluid
Conclusion: Antibody responses to PEDV were detected as early as 7 dpi and 14 dpi in serum and oral fluid respectively. Serum IgA and IgG ELISA antibodies peaked at 21 and 28 dpi and started to decline. Among the viral structural proteins, serum antibodies response against S, and N proteins was detected as early as 7 dpi followed by M and E proteins. SN activity showed up at 14 dpi spiking after rechallenge at 56 days but overall titers were low. Oral Fluid IgA and IgG ELISA antibodies peaked at 35 and 21 dpi and started to decline at different rates. IgA was major antibody isotype in oral fluid

Disclosure of Interest: None Declared

Keywords: humoral immunity, PEDV , Serum

Viral and Viral Diseases
PED

PO-PW1-050

Emergence of porcine epidemic diarrhea virus in Italy

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Introduction: Porcine epidemic diarrhea (PED) is an acute and highly contagious enteric disease characterized by severe enteritis, vomiting, watery diarrhea and high mortality in seronegative neonatal piglets. In the last years, PED has had a big economic impact on swine industry in Asia and United States. In 2014, PEDV has also re-emerged in many countries of Europe after about 20 years without PEDV circulation. In Italy, after the last epidemic wave in 2005-2006, different strains of PEDV has been circulating as sporadic outbreaks. This study reports a new epidemic wave in Italy during 2015 caused by the so-called S-INDEL variant.
Materials and Methods: Feces or intestine samples from 2488 pigs showing enteritis were collected from 463 farms during 2015. Most of the samples came from the North of Italy (i.e. the area with the higher density of pig production) and only few from the rest of the country. Mortality and clinical data were collected in 31 and 59 farms, respectively. After RNA extraction, samples were analyzed by a Real time PCR targeting PEDV/Transmissible Gastroenteritis Virus (TGEV)/Porcine Deltacoronavirus (PDCoV). S1 gene sequence was also obtained to confirm S-INDEL variant in each positive farm.
Results: PEDV was detected by Real-time PCR in 205 farms located mainly in Northern Italy and few in the Centre and South of Italy (9). Neither TGEV nor PDCoV were detected. The peak of outbreaks occurred in February-April and decreased in June-September. From October to December the incidence of outbreaks increased again with a 30% of PEDV-reinfection in previously infected farms. Among the positive farms, one was a nursery, 34 were nursery-finisher, 88 finisher, 12 farrow-to-finish and 67 grower-producer. Mortality was higher in the suckling piglets with a mean of 14%, a maximum of 50% and a minimum of 0%. Clinical symptoms were observed at all ages. S1 gene sequence was obtained from 195 farms. All the outbreaks were caused by strains showing > 98% identity with the S-INDEL variant prototype OH-851. The genetic variability among them ranged from 98.7-100%. The phylogenetic analysis showed different clusters consistent with the hypothesis that different entry events could have occurred in Italy.
Conclusion: Since January 2015, the S-INDEL PEDV strain rapidly spread in the high-density pig production area in the North of Italy. Most of the outbreaks were in grower-producer, nursery-finisher and finisher farms. Clinical signs and mortality rates were similar to those described in USA and other European countries in the same period.

Disclosure of Interest: None Declared

Keywords: Italy, PED, S-INDEL

Poster Abstracts

Viral and Viral Diseases
PED

PO-PW1-074

Effects of Different Levels of Colostrum Intake on the Severity of Porcine Epidemic Diarrhea in Piglets

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Introduction: Porcine Epidemic Diarrhea (PED) is a severe enteric disease that causes economic losses worldwide. Maternal passive immunity can prevent losses in suckling piglets during the period of immature immune system. Since sow colostrum is the most abundant and easily accessible resource of a farm. The aim of this study was to examine the effects of different levels of piglet colostrum intake on clinical illness and histopathological lesions from PED virus (PEDV) infection.
Materials and Methods: The experiment was conducted in a PEDV positive farm that practiced a feedback of PEDV infected material to sows during gestation. Twelve new born piglets from the same litter were divided into 4 groups of 3 piglets with the same weight distribution between groups. Colostrum were obtained from the sow of this litter and given to the piglets of each group with different proportions of colostrum to milk replacer. Group 1 received 100 ml of milk replacer. Group 2 received a mixture of 25 ml of colostrum/75 ml of milk replacer. Group 3 received a mixture of 50 ml of colostrum/50 ml of milk replacer. Group 4 received 100 ml of colostrum. After 5 h, all the piglets were orally inoculated with 5 ml of $1x10^5$ $TCID_{50}$/ml PEDV solution. The piglets were then fed with 200 ml of milk replacer. Fecal consistency was scored as follows: 0, solid; 1, pasty; 2, semi-liquid; 3, liquid, respectively. At 22 h post-inoculation, all piglets were euthanized and jejunum samples were collected for histopathology staining. Immunohistochemistry (IHC) staining of jejunum against PEDV was also performed. Intestinal villus heights were measured and ANOVA was used to compare the villus heights between each group.
Results: Groups 1 and 2 received an average fecal score of 3 whereas score 1 was given to groups 3 and 4. All groups developed blunt and fused villi but were most prominent and extensive in groups 1 and 2. The IHC staining revealed PEDV in jejunal villus cells of all groups. Average villus heights of groups 1, 2, 3, and 4 were 254.40 ± 47.40^a, 353.80 ± 55.10^b, 406.46 ± 15.65^b, and 415.10 ± 76.70^b um, respectively. Villus heights of group 1 were significantly the shortest. Piglets that received the highest amount of colostrum were least affected by PED, and vice versa.
Conclusion: The amount of colostrum consumed by the piglets showed a positive correlation with protection against PED losses as high colostrum intake reduced the severity of diarrhea and intestinal lesions. Thus, colostrum management to ensure the maximum intake of maternal passive immunity by piglets is crucial for PEDV infected farm. Factors that affects colostrum yield and immune components should be further studied.

Disclosure of Interest: None Declared

Keywords: coronavirus enteritis, maternal passive immunity , suckling pigs

Viral and Viral Diseases
PED

PO-PW1-070

Evaluation of Compost Bays to Test Positive for PEDv/PDCov on Clinically Active Farms

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Introduction: Compost bays that contain porcine epidemic diarrhea virus (PEDv) and/or porcine delta coronavirus (PDCov) positive pigs carry the risk of contaminating tractor buckets, staff shoes, saw dust delivery trucks, emptying equipment, and may allow for viral survivability on a site after herd has fulfilled the case definition of weaning negative pigs for these diseases. In addition, compost may provide fomite transmission via vultures and other carrion can potentially remove PEDv positive tissue and carry the virus to another facility. However, little documentation has been provided on the risk associated with the compost facilities and what areas of the compost are the present risk.
Materials and Methods: Four farms (divided into farms that recently (< 2 months) became PEDv/PDCov negative or farms currently weaning PEDv/PDCov positive pigs) were evaluated to determine the risk of PEDv or PDCov being present in compost bays at different time post-infection in the fall of 2014. Four foot long grain sampling probes were purchased for the study. Probes were inserted into the older compost bays (bays that have been closed for approximately 2 months). Core samples at various points along the grain probe were pulled with gloved hands (6", 12", 24", and 48"). Three core samples per bay were pooled into a Ziploc bag to represent the sample at a given depth. This was then repeated on bays that were currently used for farm mortalities. The probe was then cleaned and stored as some farms were tested multiple times. Samples were submitted to the University of Minnesota Diagnostic Laboratory for PCR RNA analysis of PEDv and PDCov. Data were reported as percentages of samples with each facility site, herd status, and PCR result category (positive, negative, or suspect).
Results: No PCR positive samples were found in the testing that was done. There were suspect cases of PDCov discovered in the older bays on separate farms on different days; however, it is not determined if these were false positives as bioassays were not performed.
Conclusion: Overall, the incidence rate of finding virus in the compost bay appears to be low when temperatures in the compost remained above 140˚F.

Disclosure of Interest: None Declared

Keywords: Compost, PEDv

Viral and Viral Diseases
PED

PO-PCO1-004

Impact of Porcine Epidemic Diarrhea Virus (PEDV) subunit vaccine in endemically infected breeding herd in North America.

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Introduction: The objective of this study was to evaluate the efficacy of a sub-unit PEDV vaccine (Harris Porcine Epidemic Diarrhea Vaccine, RNA®) in an endemically infected breeding herd that had experienced elevated pre-weaning mortality (40-60%) for 26 weeks.
Materials and Methods: On May 4, 2014 a 2,500 head breeding farm reported watery diarrhea and vomiting in 7 day old piglets. Samples were negative for TGE, but positive for PEDV by PCR. Sequencing of the S1 region (2.2kb) indicated it was 99% similar to the strains circulating the U.S. Whole herd feedback (affected piglet intestines) was initiated and all adult animals on the farm received the inoculum twice in the first week of the outbreak. No gilts were entered for 6 months and were given the same oral PEDV inoculum (feedback) 10 weeks prior to entry to the breeding herd. All pigs older than 12 days were weaned early and any new pigs being born were euthanized for a 3 week time period. McREBEL movement restrictions were put into place. All farrowing rooms were cleaned, disinfected and dried prior to loading new sows. Despite these changes, the herd continued to see PEDV related diarrhea at 2-3 days of age and pre-weaning mortality continued at 40-60% even out to 6 months later. On October 29th, the farm initiated a single pre-farrow vaccination program, where dams were vaccinated with the PEDV vaccine 7-14 days prior to farrowing (per label).
Results: The first week the vaccinated dams farrowed, a dramatic reduction was seen in both % of litters showing clinical signs as well as pre-weaning mortality during the first 7 days. The first group of pigs weaned from vaccinated dams saw a 200% reduction in pre-wean mortality from the week prior. Within 5 weeks, the farm could no longer find evidence of PEDV infection by PCR on suckling piglets that were being tested on a weekly basis (n=20). A statistical analysis, using a GLM procedure in ANOVA, was run comparing the 14 weeks prior to the initiation of the vaccine program (control) to the 14 weeks following initiation of the vaccination program (treatment). With each group having approximately 1400 litters, the pre-weaning mortality of the control group was 64.4% and the pre-weaning mortality of the treatment group was 23.1% ($p<.001$). Baseline pre-wean mortality prior to break was 18%.
Conclusion: Despite extreme efforts in feedback, cleaning and sanitation, clinical signs and elevated mortality persisted in this endemically infected breeding herd. It wasn't until a sub-unit vaccine was applied in a pre-farrow protocol, with intent to booster secretory IgA, was there a significant reduction in clinical signs and mortality in suckling pigs.

Disclosure of Interest: None Declared

Keywords: PEDV, vaccine

Viral and Viral Diseases
PED

PO-PW1-059

Lymphoproliferative responses in mesenteric lymph node of pigs inoculated with either classical or pandemic PEDV variant

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Introduction: Porcine epidemic diarrhea (PED), a devastating enteric disease, has caused severe economic losses to swine industry worldwide since its emergence in 1977. Due to PED virus (PEDV) causing enteric infection, local immunity including secretory IgA is more important for protection compared to systemic immunity. Cellular immunity also plays an important role in the protection and disease recovery. The objectives of the study were to investigate cellular response in mesenteric lymph node following oral exposure either classical or pandemic PEDV variant.
Materials and Methods: Eighteen, 3-weeks-old pigs were randomly allocated into 2 groups of 9 pigs each, including Classical and Pandemic groups. Pigs in classical and pandemic groups were orally challenged with 2 ml of 10^4 $TCID_{50}$/ml of either classical or pandemic PEDV variant. Three pigs per group were randomly selected and necropsied at 3, 7 and 14 day post infection (dpi). Mesenteric lymph node was collected and mononuclear cells (MNCs) were isolated and lymphocyte proliferation assay (LPA) was performed using carboxyfluorescein diacetatesuccinimidyl ester (CFSE) assay. *In vitro* stimulation included mock, PHA, classical or pandemic PEDV variant at MOI of 1. Stimulating index (SI) was calculated and subgroups of MNC stimulation consisting of classical inoculated - classical stimulated (CI-CS), classical inoculated - pandemic stimulated (CI-PS), pandemic inoculated - pandemic stimulated (PI-PS) and pandemic inoculated - classical stimulated (PI-CS).
Results: The specific lymphocyte proliferation was first detected at 3dpi and maintain until the end of study (except group PI-CS decrease in 14 dpi). The peak of SI was observed at 3 dpi, 14 dpi, 3 dpi and 7 dpi in group CI-CS (3.29), CI-PS (2.02), PI-PS (1.32) and PI-CS (1.31), respectively. Especially, SI of group CI-CS at 3 dpi was significantly higher than other group whereas SI in group PI-CS was significantly lower than group CI-CP and CI-PS. However, no difference of SI between groups at 7 dpi.
Conclusion: Based on lymphoproliferative responses of mesenteric lymph node, response of classical variant inoculated pigs were relatively higher than that of pandemic variant inoculated pigs. Response against homologous stimulation was relatively higher than that of response against heterologous stimulation in both inoculated groups.

Disclosure of Interest: None Declared

Keywords: lymphocyte proliferation, mucosal immunity, porcine epidemic diarrhea

Poster Abstracts

Viral and Viral Diseases
PED

PO-PW1-034

The Correlation between Sows and Their Piglets Relating to Immunity Against the Porcine Epidemic Diarrhea Virus

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Introduction: Porcine Epidemic Diarrhea (PED) is caused by PED virus (PEDV) that is a member of the order *Nidovirales*, family *Coronaviridae* and genus *Coronavirus*. PED is a severe enteric disease that causes economic losses worldwide. Maternal passive immunity can prevent losses in suckling piglets during the period of immature immune system. Sow colostrum is the most abundant and easily accessible resource of a farm. The aim of this study was to evaluate an immunological correlation between sow and their offspring.
Materials and Methods: Forty three sows within parities 1, 3 and 5 were selected from 4 different Thai pig farms; a PEDV free farm (A), a farm which was previously infected with PEDV and PED vaccination was applied in sows (B) and two farms which were previously infected with PEDV and feedback technique for PEDV was applied in sows at 4 weeks before farrowing (C and D). Blood and colostrum samples were collected from these sows at parturition as well as blood samples of their piglets at 5 days old. Sow's blood samples were collected from jugular veins whereas colostrum samples were collected from the first teat within 30 min post-parturition. All samples were analysed for specific PED immunoglobulin A (IgA) and immunoglobulin G (IgG) using an in-house PED ELISA test kit which was developed by the Diagnostic Unit, Faculty of Veterinary Medicine, Kasetsart University, Kamphaeng Saen campus, Thailand. The data was analysed the immunity correlation by using statistical R-program.
Results: For farm A, very low S/P ratios (negative results) of both IgG and IgA in sow's colostrum and serum were detected. The S/P ratios of IgG in serum and colostrum in vaccinated herd were higher than those in feedback herds. On the other hand, feedback herds had higher S/P ratios of IgA in serum and colostrum. As a result, the S/P ratios of IgG and IgA between sow serum and colostrum showed a positive correlation (r=0.75 and 0.61, respectively). In addition, sow colostrum IgA showed S/P ratio approximately 1.5-2.0 times more than S/P ratio of piglet's serum IgA at 5 days old. The S/P ratios of IgG and IgA in sow's colostrum were found to have a positive correlation to those in piglet's serum (r= 0.72 and 0.71, respectively).
Conclusion: The in-house PED ELISA test kit can be applied to check IgA and IgG in serum and colostrum. The feedback technique induces the higher level of IgA in colostrum. Piglets from sows with high PED immunity in colostrum also have high PED titer in serum. In conclusion, endemically PED-infected farms must immunize with a suitable technique for a high level of immunity against PEDV and they should focus on piglet colostrum intake management.

Disclosure of Interest: None Declared

Keywords: Correlation, PEDv, pig immunity

Viral and Viral Diseases
PED

PO-PW1-038

Different capabilities of five ELISAs for detection of antibodies against PEDV in pigs exposed to geographically different strains

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Introduction: Recently, PEDV has caused severe economic losses to the swine industries in the Americas and Asia and has now also been reported in Europe. Reliable serological assays are essential for epidemiological studies and vaccine evaluation. It has been suggested that ELISAs based on the whole virus (WV) antigen or the nucleocapsid protein (NP) would be more sensitive for detection of antibodies against heterologous PEDV strains than ELISA based on the spike 1 (S1) protein. The objective of this study was to assess the diagnostic performance of a commercial and four *in-house* ELISAs based on PEDV the WV, the NP protein or the S1 protein.
Materials and Methods: A total of 733 serum samples from North American or European pigs with known (n=380) or unknown (n=353) PEDV exposure status were tested with each of three indirect ELISAs (NP1-I, NP2-I, S1-I), a blocking ELISA (WV-B) and a competitive (NP-C) ELISA. Specifically, 86 samples were obtained from pigs experimentally infected with genogroup 1 (G1), experimentally infected with genogroup 2 US prototype (G2) or S INDEL (G2-INDEL), or sham infected. Additional 149 samples from farms exposed to PEDV and 100 samples from farms with no exposure to PEDV were obtained from Italy. Furthermore, 45 samples positive to transmissible gastroenteritis virus (TGEV) or porcine respiratory virus (PRCV) were used. Finally, 353 porcine serum samples with unknown PEDV exposure status originating from the US or from Italy were also included.
Results: Overall, all five evaluated tests had a moderate agreement (κ = 0.61). All assays correctly identified pigs infected with G1, G2 or G2-INDEL. G1 infected pigs were earliest detected by the S1-I ELISA, G2-INDEL infected pigs were earliest detected by the WV-B ELISA and the NP-C ELISA, and the performance of all tests was similar for the G2 group. The WV-B ELISA presented the overall highest number of positive samples (48%, 315/647) and the NP1-I ELISA and NP-C ELISA presented the lowest detection rates (175 and 179/647, respectively) (p <0.01). WV-B and NP1-I ELISAs detected 2/21 and 1/21 TGEV antisera respectively, and NP1-I detected an additional 1/24 PRCV antisera.
Conclusion: The NP1-I had the overall lowest detection rates from sample subsets derived from both experimentally and naturally infected animals. The WV-B had the overall highest detection rates. Differences in detection rates among assays seem to be more related to intrinsic factors of an assay than to the PEDV antigen used. The decision which assay should be used for PEDV antibodies monitoring at the herd level ultimately needs to be based on desired specificity and sensitivity levels, easiness of testing, and access to a certain assay.

Disclosure of Interest: P. Gerber: None Declared, D. Lelli: None Declared, J. Zhang: None Declared, B. Strandbygaard : None Declared, S. Perulli : None Declared, A. Bøtner : None Declared, L. Comte Conflict with: IDvet, M. Roche Conflict with: IDvet, P. Pourquier Conflict with: IDvet, T. Opriessnig: None Declared

Keywords: Antibodies, Diagnostic test, PEDv

Viral and Viral Diseases
PED

PO-PW1-054

The emergence of porcine epidemic diarrhea virus in United States is associated with its outbreak in China, 2013

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Introduction: Porcine epidemic diarrhea virus (PEDV) belongs to genus alphacoronavirus. The disease, PED, which causes high mortality rates in newborn piglets, is characterized by acute vomiting and watery diarrhea. During late 2010 in China and Southeast Aisa, several PEDV strains were initially isolated. Subsequently, the disease was pandemic in several provinces neighboring Zhejiang and later in United States in 2013. It aroused our great interest to analyze the reasons behind the outbreak.
Materials and Methods: A total of 169 fecal and intestinal samples were collected from pigs with typical PED symptoms on 26 farms in 4 provinces neighboring Zhejiang during January 2012— July 2013. Reverse transcription PCRs (RT-PCR) specific for spike (S) gene were performed. Based on the PEDV MN strain(GenBank accession No. KF468752), all primers were designed. Then TA cloning were conducted and Vector NTI software was used to assemble and analyze the sequences. Multiple alignments of sequences measured with available sequences from Asia and United States were performed, and then phylogenetic analyses using MEGA 5.2 program. Recombination events were identified by 6 methods (Recombination Detection Program, GENECONV, BOOTSCAN, MaxChi, CHIMAERA and SISCAN) and MN strain was used as a query. The major recombination breakpoints were detected by bootstrap analysis.
Results: The detection rate was 56.8% (96/169). Among 24 representative samples, the sequences of the full length genomic cDNA of the strain CH/ZJCX-1/2012, the S gene of ZJQZ-2w/2012 and CH/ZJDX-1/2012, and the region encoding structural protein genes by an order of 5'-S-ORF3-E-M-N-3' (5'-spike protein-open reading frame 3-envelope-membrane-nucleoprotein-3') of the remaining strains were detected. All the strains isolated were in G2 genogroup, unlike late 2010 isolated in G1 genogroup. Among these, the AH2012 strain was clustered closly with the U.S strains in the ORF1ab and the N gene region, unlike the ZMDZY sublineage in the S-ORF3-E-M region. Two regions of putatively major recombination breakpoints were detected: one covered the 3' half of ORF1a, complete ORF1b, and the N terminus of S, the other spanned partial S, ORF3, E, M, and partial N between the AH2012 strain and ZNDZY sublineage.
Conclusion: It is possible that replacement of a region within the partial S-ORF3-E-M-partial N region of the AH2012 strain with the corresponding fragment close to the ZMDZY sublineage resulted in a recombinant strain related to the outbreak of the virus in swine in eastern China and emergence in the U.S, 2013. Other unidentified recombination events and accumulation of adapted mutations within the structural protein genes were also likely involved in this process.

Disclosure of Interest: None Declared

Keywords: outbreak, porcine epidemic diarrhea virus (PEDV), recombination

Viral and Viral Diseases
PED

PO-PW1-073

Preliminary results on the time-to-baseline-production and total loss in Latin American farms after PEDv introduction

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Introduction: The speed and discipline with which strategies to control porcine epidemic diarrhea virus (PEDv) are implemented in a production system are important from a financial standpoint, as PEDv-positive flows have worse throughout, growth and feed efficiency. Recently, Linhares et al 2013 developed a method to quantify losses as well as speed of recovery with regards to piglet throughput before PRRSv introduction in sow farms. The objective of this study was to quantify the speed of recovery and measure total production loss in terms of piglets not weaned due to PEDv in Latin American breeding herds undergoing virus control and elimination.
Materials and Methods: Production records containing number of pigs weaned per week from 24 pig farms that had no previous history of PEDv were obtained. Records contained data before and after PEDv detection. Previously described statistical process control (SPC) methods were used to calculate time-to-baseline-productivity (TTBP) and total piglets not weaned/sow (Total Loss) for each herd (Linhares & Morrison, 2014).
Results: The average (and range) sow herd size of participating farms was 2,834 (200 to 12,000). There were 23 farrow-to-wean farms and 1 farrow-to-finish farm. Seven farms had batch farrowing production compared to the rest which weaned pigs on a weekly basis.
The overall average TTBP was 10 ± 4.6 ranging between 6 and 22 weeks. Large sow herds (i.e. ≥ 1000) compared to small herds had a TTBP of 12 and 7.8, respectively. Most of the small herds were under a batch farrowing production system; however, there was no difference in TTBP when weekly and batch farrowing production farms were compared. The overall average total per sow was 2.65 ± 1.72 ranging between 0.8 and 8.1. The correlation between TTBP and total loss was 0.44.
Conclusion: The TTBP for Latin American farms undergoing PEDv control and elimination was similar to what has been previously reported by Goede and Morrison in 2015 on US herds. An important variation was found on TTBP. We believe that farms lacking implementation consistency of recommended practices for PEDv control and elimination took longer to reach TTBP. There were 3 farms in the database that never reached TTBP. Specifically, these farms continued to feedback more times after the initial feedback or moved to a pre-farrow feedback which did not occur in the other farms. The lack of association between production system (batch vs weekly farrowing) and TTBP may be explained by the low number of observations. In this study, TTBP and total loss revealed a high variability component among farms which may be explained by the way the control and elimination procedure is conducted.

Disclosure of Interest: None Declared

Keywords: Baseline production, PEDv, TTBP

Poster Abstracts

Viral and Viral Diseases
PED

PO-PW1-051

Addition of bovine plasma into the diet decreases PEDV shedding and increases IgA responses in experimentally infected pigs

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Introduction: Plasma of porcine origin often contains PEDV RNA which raises some concerns about biosecurity and transmission of viruses within pig populations. In contrast, it is well recognized that the addition of plasma to pig feed enhances immune reactions and also has some intrinsic inhibition on virus survival. The objective of this study was to determine if there is any benefit to the diet containing spray-dried plasma (SDP) of bovine origin during acute PEDV infection.
Materials and Methods: Three groups of five 3-week-old conventional pigs were used. For the inoculation, commercial raw porcine plasma positive for PEDV neutralizing antibodies and PEDV RNA (PORCINE-RAW-PLASMA) was used and was either left unspiked (negative control group) or was spiked with a PEDV stock and stored at 19°C for 1 h. The negative control group received 10 ml unspiked PORCINE-RAW-PLASMA orally, the PEDV group received 10 ml PEDV-spiked PORCINE-RAW-PLASMA orally, and the PEDV-bovine-SDP group received 10 ml PEDV-spiked PORCINE-RAW-PLASMA orally and was also fed SDP of bovine origin at 5% of the ration for the study duration. Fecal swabs were collected every day and tested for presence of PEDV RNA. Serum samples were collected at inoculation (dpi 0) and at dpi 7 and 14 and tested for PEDV IgA and IgG antibodies. Necropsy was done on dpi 14 and intestinal sections were collected and microscopically evaluated for PEDV lesions and antigen by IHC stains.
Results: PEDV was not detected in the negative control group. In the PEDV and the PEDV-bovine-SDP groups, clinical signs were mild; a few pigs in both groups developed mild-to-moderate diarrhea or vomiting for 1-3 days. The average fecal PEDV RNA shedding time ± SEM was 7.2±1.0 days for the PEDV-bovine-SDP group and 9.4±1.7 days for the PEDV group. While PEDV RNA was no longer detectable after day 11 in the PEDV-bovine-SDP group it was still present up to termination of the study at day 14 in the PEDV group. By day 7, 3/5 PEDV-bovine-SDP pigs had IgA antibodies whereas 0/5 pigs of the PEDV pigs were IgA positive. By day 14 all pigs in the PEDV and PEDV-bovine-SDP groups were IgA and IgG positive. One PEDV pig had moderate enteric lesions at day 14 and PEDV antigen was present in enterocytes in the small intestines of that pig.
Conclusion: The results of this study indicate a positive effect of bovine SDP on acute PEDV infection as the addition of bovine SDP to the diet resulted in a faster IgA response and also reduced the PEDV shedding time compared to pigs that didn't receive bovine SDP. This could be important for disease outcome and transmission. Furthermore, the commercial raw porcine plasma used for inoculation didn't contain infectious PEDV.

Disclosure of Interest: None Declared

Keywords: Bovine Plasma, Experimental Infection, PEDV

Viral and Viral Diseases
PED

PO-PW1-045

Characterization of a French Indel strain of porcine epidemic diarrhea virus isolated in December 2014

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Introduction: Porcine epidemic diarrhea (PED) characterized by watery diarrhea and vomiting, was described throughout Europe till the end of the 1990's and is caused by an Alphacoronavirus, the PED virus (PEDV). Since April 2013, a severe epizooty of PED has been striking USA previously free from this disease. Suckling piglets are the most affected by PED with up to 90-95% mortality. In China in 2010 and USA in 2013, PED epidemics were related to new PEDV strains. Today, two types of PEDV strains circulate in those countries, non-InDel and Indel strains. Since 2014, new outbreaks have been reported in Europe and only associated to InDel strains. In France PED is notifiable as emerging disease. An outbreak was reported in North of France in December 2014. The objective of the study was to characterize the PEDV isolate in the French PED outbreak, to evaluate the duration of viral shedding in feces and to compare experimentally the pathogenicity of this strain with the old European reference PEDV strain, CV777.
Materials and Methods: The outbreak occurred in a farrow-to-finish herd located in the North of France in December 2014. Jejunum from 3 affected animals which had died within the day were sampled and used to extract the PEDV genomic RNA to determine the whole genome sequence using next-generation sequencing. RT-qPCR targeting the N gene was performed on feces sampled every 2 weeks for 2 months to assess the duration of viral shedding in the herd. The PEDV strain was used to inoculate orally three-week old specific-pathogen-free (SPF) piglets to compare the pathogenicity of this strain to the CV777 PEDV reference strain.
Results: PEDV infection was confirmed in this herd from North of France in December 2014. The strain named PEDV FR/001/2014 is genetically related to the German GER/L00719/2014 PEDV strain (99.9% of identity) isolated in May 2014 and belongs to the Indel PEDV strains. The virus was shed in feces for 29 days in affected suckling piglets and 20 days in fattening pigs. Clinical signs typical of PED were reproduced experimentally in 3 week-old SPF piglets using FR/001/2014 PEDV strain. The virus was transmitted in one day by direct contact to other piglets. A viremia was observed in FR/001/2014-inoculated piglets compared to CV777-inoculated piglets.
Conclusion: In conclusion, the FR/001/2014 PEDV strain was close to an Indel strain isolated in Germany few months earlier. Viral shedding decreased rapidly in PED affected animals. The virus was not transmitted to other herds. Finally, PED was experimentally reproduced in SPF piglets inoculated orally with the FR/001/2014 PEDV strain.

Disclosure of Interest: None Declared

Keywords: diarrheal viruses, emerging porcine viruses

Viral and Viral Diseases
PED

PO-PW1-036

Diagnosis of PEDV in Colombia Swine Farms

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Introduction: Porcine Epidemic Diarrhea Virus (PEDV), a member of the family Coronaviridae genus Alphacoronavirus is an enveloped, single – stranded RNA virus. PED was first reported in Europe in 1978. Since then outbreaks of PED infections have been reported in many countries including Europe and Asia. In America, the PEDV was first identified in USA in May 2013.The purpose of this study was to report the detection of PEDV in natural outbreaks in Colombian Swine Farms.
Materials and Methods: Samples from 186 pig farms in Colombia from March 2014 through December 2015 were submitted to the National Veterinary Laboratory of ICA. All of the pig farms had similar disease histories, clinical signs, and lesions, including the presence of a sudden outbreak and rapid spread between farms, high mortality in neonatal piglets. Affected piglets displayed anorexia, vomit, diarrhea, and dehydration. Clinical specimens were collected and tested for porcine epidemic diarrhea virus including feces and tissues. Samples were tested by real time reverse transcriptase PCR (rRT-PCR) as described for the nucleoprotein (N) gene. Formalin fixed intestinal tissues were also examined by histopathology and the PEDV antigen was detected by immunohistochemistry using a monoclonal antibody specific for the nucleoprotein as described. Samples from 10 cases were sent to NVSL USDA to reconfirm our diagnosis and also to conduct preliminary sequencing.
Results: A total of 149 pig farms were confirmed as positive for PEDV by qPCR. Microscopic intestinal lesions consistent with viral enteritis were observed in all cases examined. The PEDV antigen was found in the cytoplasm of villous enterocytes in acute cases studied. Ten PEDV strains sequenced in NVSL were found 99 to 100% identical to the NPL-PEDV USA isolates detected in 2013 and 2014.
Conclusion: Our data demonstrated that PEDV is now endemic in Colombia. So far the virus has been detected in 10 departments of the main pig production regions.PEDV isolates are genetically similar to US isolates. These strains were responsible for the recent PEDV outbreak in our country and they produced a similar mortality and pathological findings as reported for the US isolates. The source of this emergent PEDV in Colombia is still unknown.

Disclosure of Interest: None Declared

Keywords: Colombia, Diagnosis, PEDV

Viral and Viral Diseases
PED

PO-PW1-079

Experimental infection of piglets with an early European strain of PED virus and a recent US PEDV strain

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Introduction: Outbreaks of porcine epidemic diarrhoea (PED) were reported across Europe during the 1980's and 1990's but only sporadic outbreaks occurred in recent years. PED virus (PEDV) spread for the first time into the USA in 2013 and has caused severe economic losses. Retrospectively it was found that two different strains of PEDV have been introduced into the US, both are closely related to strains circulating in China where a new wave of the disease occurred from 2010 onwards. Since autumn 2014, new outbreaks of PED have occurred in Europe.
Materials and Methods: In this study, weaned piglets were inoculated with an early European isolate (Br1/87) or faecal/intestinal suspensions derived from pigs infected with a recent European strain of PEDV (from Germany) or a US strain of PEDV.
Results: No evidence for infection resulted from inoculation of pigs with the German sample that contained high levels of PEDV RNA; there were no clinical signs, excretion of viral RNA or anti-PEDV antibody production. Mild clinical signs of infection, mainly diarrhoea, occurred in piglets inoculated with the Br1/87 and US PEDV strains. PEDV RNA was detected throughout the intestine at 4 days post-inoculation. In addition, low levels of viral RNA were detected in lungs and livers with higher levels in spleens. Seroconversion against PEDV occurred in infected animals within 10 days. PEDV RNA excretion occurred for at least 2 weeks. The US PEDV RNA was detected at low levels in serum samples on multiple days. Current diagnostic systems can detect infection by the different virus strains.
Conclusion: Infection of piglets by the early European isolate of PEDV (Br1/87, a close relative of the CV777 strain) and by a US non-INDEL strain of the virus has been performed and it has been possible to monitor infection by each strain using a range of diagnostic assays for the presence of viral RNA and the induction of anti-PEDV antibodies. The mild clinical outcome observed in this study may be related to the age of piglets used. Newborn piglets appear most severely affected by the infection in the field and hence experimental infection of such piglets can be expected to produce a more severe disease and even mortality. Indeed, the outcome of PEDV infections in pigs appears to be dependent upon a range of factors (see EFSA AHAW panel report, 2014).

Disclosure of Interest: None Declared

Keywords: clincal signs, seroconversion, virus excretion

Poster Abstracts

Viral and Viral Diseases
PED

PO-PW1-035

Stable Expression and Purification of the Ectodomain of Porcine Epidemic Diarrhea Virus (PEDV) Spike (S) Protein

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Introduction: Since late 2010, new variants of porcine epidemic diarrhea virus (PEDV) have arisen in China, spread to Asia and North America in the end of 2013, and cause severe watery diarrhea in pigs of all ages and extremely high mortality rate in neonatal piglets resulting in significant economic losses. Recent studies indicate that the spike (S) gene of Taiwan new PEDV strains are closely related to global non-S INDEL PEDVs but significantly different from historic and traditional PEDV vaccine strains, that might explain the failure of cross protection of current available vaccines in pigs. The S protein is considered as a promising candidate for development of a subunit vaccine against PEDV. We present the development of a stable expression system using mammalian cells for production of full-length ectodomain of S protein from a non-S INDEL PEDV isolated from Taiwan.

Materials and Methods: To establish the S protein expressing stable cell line, sequence of S protein of a Taiwan non-S INDEL strain was cloned into pcDNA 3.1/V5-His-TOPO® vector. After transfecting the vector into HEK293 cell line for 48 hrs, the cells were selected by G418 for more than 2 weeks to generate a stable 293 cell line expressing PEDV S protein. The recombinant S protein was further purified by metal-binding affinity of histidine (TALON Superflow, GE healthcare), desalted by PD-10 desalting columns (GE healthcare), and concentrated by Vivaspin™ (GE healthcare). The expression and the size of the expressed PEDV S protein were confirmed by the western blot and immunocytochemistry staining (ICC) using anti-V5 (invitrogen) and anti-PEDV monoclonal antibodies.
Results: A 293 cell line stably expressing the PEDV S protein has been successfully established. The expressing of the PEDV S protein was detected in more than 60% cells of the stable cell line for more than 20 passages by ICS using anti-V5 monoclonal antibody. By western blot assays, the size of the S protein, about 180kDa, was detected and was consistent with the predicted size of the protein. The expressed S protein was also further characterized and confirmed by anti-PEDV S monoclonal antibodies to prove the recombinant PEDV S protein exhibiting similar biological nature as the PEDV S protein.
Conclusion: The PEDV S protein is majorly responsible for viral entry via interactions with specific host cell receptors and for induction of neutralizing antibodies. The PEDV S protein from the stable 293 cell line may be a promising candidate for development of a subunit vaccine or diagnostic ELISA assays for global PEDVs.

Disclosure of Interest: None Declared

Keywords: Porcine epidemic diarrhea virus, spike protein, subunit vaccine

Viral and Viral Diseases
PED

PO-PW1-066

Identification of Porcine Epidemic Diarrhea virus (PEDV) associated with the 2014 outbreak in Mexico.

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Introduction: During 2013 an outbreak of porcine epidemic diarrhea virus in the United States drew attention of all those related to the swine industry, causing up to 100 % mortality in piglets. In Mexico, in March 2014 the first outbreak of PED was detected in the State of Mexico with 100 % mortality in piglets. The aim of this study was to confirm and identify the PEDV, from samples of piglets with suggestive signology using molecular techniques and electron microscopy.
Materials and Methods: Euthanasia and necropsy was performed and samples obtained where lung (13), stomach content (13), stomach (13), small intestine (8), and feces (15). Fragments of small intestine were fixed in 2.5% glutaraldehyde for 24 hours, washed, postfixed with osmium tetroxide 1% and washed again. Then, dehydrated with increasing concentrations of acetone and embedded in epoxy resin. Semi-thin sections (200 nm) were cut, mounted on slides and contrasted with toluidine blue. Fine cuts of 60 nm were performed, mounted on copper grids, contrasted with uranyl acetate and lead citrate and visualized in a Zeiss EM 900 electron microscope. Along with the electron microscopy, the presence of virus in clinical samples was confirmed by RT-PCR, amplifying the S gen. RNA extraction was performed with Invitrogen TRIzol® Reagent and the RT-PCR with QIAGEN® OneStep RT-PCR kit. The products are placed in a 2% agarose gel and purification was done using “E-Gel SizeSelect” (Invitrogen). The products were sequenced to confirm their identity in an ABI 3130 sequencing platform. The sequences were edited, assembled and aligned by Jalview, Multalin 5.4.1, and MEGA6.
Results: Macroscopic and microscopic lesions were suggestive of acute infection with Porcine Epidemic Diarrhea. Numerous spherical viral particles were observed in the electron microscopy. The morphology and size of the viral particles coincides with the characteristics of coronavirus. Of a total of 62 samples, 32 were positive (51.61%), 9 lungs (69.23%), 13 of stomach content (100%), 4 stomach (30.76%), 5 small intestine (62.5%), 1 feces (6.66%). The sequences obtained for the S gene showed 99% similarity to the same region of the NPL-PEDV/2013/P10 strain (KJ778616), with the strain YY K13JA11-4 (KJ539153), and the OH14 (KJ408801) strain.
Conclusion: This study confirms the virus characterization of PEDV by amplification of a fragment of the glycoprotein S gene and confirmed by sequencing (GenBank No. KM044331), as well as the coronavirus-like particles found in the tissues studied. The nucleotide sequence analysis of the isolated virus of the 2014 outbreak in Mexico showed a marked homology to viruses that circulated in 2013 in Colorado, USA.

Disclosure of Interest: None Declared

Keywords: Electron Microscopy, PCR, PED

Viral and Viral Diseases
PED

PO-PW1-032

PEDV exposure protects pigs against homologous re-exposure 44 days later

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Introduction: PEDV emerged in the US during 2013 and rapidly spread from farm to farm causing high morbidity and mortality resulting in high economic losses to the US swine industry. As the virus made its way through swine dense populations there were many questions on degree and length of protection after initial exposure. The objective of this study was to determine if initial expose of a group of pigs to a PEDV genogroup 2 prototype strain induced homologous protection against re-challenge.
Materials and Methods: Sixty 10-day old pigs were used; 32 pigs were orally infected with PEDV Co-13 at 10 days of age (=day 0). All sixty pigs were orally infected with PEDV Co-13 44 days later (=day 44) when the pigs were approximately 8 weeks old. Fecal swabs were collected daily and tested by a PEDV real-time PCR for PEDV RNA. In addition, serum was collected at day 0, 7, 14, 24, 44 and day 58-60 to test for PEDV-specific IgG and IgA by ELISA. Pigs were randomly selected for necropsy at 3, 14, or 16 days after re-challenge.
Results: After initial infection at day 0, pigs started to shed PEDV in feces from day 1 onwards. Essentially all samples from all infected pigs were positive until day 8 at which time shedding became intermittent but continued over the following weeks. Two of the 32 pigs were still PCR positive on feces by day 44. PEDV IgA antibodies were detected in two infected pigs by day 7 and most infected pigs were IgA positive by day 14. IgA levels were still high at the time of re-challenge. Six of the 32 pigs had detectable IgG levels by day 7 and 19/32 were IgG PEDV positive at day 21. At day 24 and 44 all pigs were IgG positive. After re-challenge, PEDV RNA was detected in a fecal sample from one pig whereas the majority of the pigs without previous PEDV exposure were PCR positive by day 2. After re-challenge randomly selected pigs in both groups were necropsied at 3, 14 or 16 days post challenge. Lesions or PEDV antigen were not detected in any of the exposed pigs whereas naïve pigs had moderate lesions in enteric sections associated with moderate to high amount of PEDV antigen.
Conclusion: Results indicate that previous expose to PEDV can induce protective homologous immunity within 44 days providing insight to the duration of immunity.

Disclosure of Interest: None Declared

Keywords: Experimental Infection, porcine epidemic diarrhea virus (PEDV), Re-exposure

Viral and Viral Diseases
PED

PO-PW1-080

An evaluation of the immune response induced by the PEDV conditional vaccine on animals that were naturally exposed to wild virus 18 months prior.

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Introduction: The object was to determine if Zoetis' conditionally licensed PEDV vaccine will enhance immunity in animals that were exposed to wild PEDV virus approximately 18 months prior.
Materials and Methods: This project was performed from 7/20-9/8 2015 at a 2,200 sow breed-to-wean site that broke with PEDV in February 2014. The enrolled sows farrowed in August 2015 which is approximately 18 months post-infection. The study's protocol used only females present during the outbreak of PEDV, so parity 3 and older females were selected for the study. Enrolled females were randomly assigned to one of the following two treatment groups. Treatment group 1 was control and treatment group 2 was vaccinated with one dose two weeks pre-farrowing. Each group consisted of 30 total females. Samples were collected from at least 16 animals. The reason for fewer animals then enrolled was due to proper timing of colostrum collection. Treatment groups were bled at two weeks pre-farrow. Colostrum was collected at farrowing and milk was collected at five days post parturition. Samples were tested at South Dakota State University for the FFN/serum neutralization test. Treatment groups were evenly distributed over two "breeding" weeks due to a decision by the farm's owner to insert a higher percentage of replacement gilts.
Results: The presence of PEDV was not detected on routine tissue and rectal swab submissions prior to starting the trial. The objective was to make sure an endemic situation was not present. The conditional PEDV vaccine had been utilized in the sow herd since November 2014 as a single dose prefarrowing. The females enrolled into the trial had been vaccinated with the vaccine prior to each previous farrowing with the last vaccination approximately four month previously. Four different charts will illustrate the outcomes of this study. One chart shows the baseline serum neutralizing antibody statue prior to vaccination. A second chart will illustrate treatment 2 response in serum antibodies compared to non-vaccinates. A third chart will illustrate no difference between vaccinates and non-vaccinates in neutralizing antibodies in colostrum, although both groups had high titers. A final chart will show higher and more uniform neutralizing antibody titers in milk samples.
Conclusion: In conclusion animals that had experienced wild virus exposure even up to 18 months prior, the use of the Zoetis conditional PEDV at two weeks pre-farrowing, resulted in a two-fold serologic response at the time of farrowing and resulted in higher and more uniform neutralizing antibody titers in milk (lactogenic immunity).

Disclosure of Interest: None Declared

Keywords: 18 months, lactogenic immunity, PED

Poster Abstracts

Viral and Viral Diseases
PED

PO-PW1-060

Molecular and biological characterization of porcine epidemic diarrhea virus isolates detected in Ukraine

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Introduction: Porcine epidemic diarrhea virus (PEDV) is a member of the family Coronaviridae that causes severe diarrhea, vomiting, and dehydratation. PEDV can infect pigs of any age, however high mortality rates are commonly observed in newborn piglets. This virus was first observed in Belgium in 1971, and during the next few years PEDV spread across the Europe. By 2013 disease was believed to be restricted to Asian countries. However, later that year some outbreaks of infection were reported in the USA and Europe. Also in summer of 2014 outbreak was registered in Ukraine. The aim of the study was to analyze the molecular and biological features of PEDV isolates detected in Ukraine.
Materials and Methods: Viral RNA was extracted using «MagVet™» Universal Purification Kit. Reverse transcription was performed with a commercial kit REVERTA-L (AmpliSens). Detection of the virus was carried out with an in-house nested RT-PCR kit. Full-length genome sequencing of detected virus isolate was performed by UK Animal and Plant Health Agency (Karr, 2014). The sequence was compared with other strain of PEDV from the USA, Asia and Europe. Phylogenetic analysis was done by using MEGA 6 software. The tree was constructed by using the neighbor-joining method. The intestinal tissues of infected pigs were analyzed to investigate the pathomorphological changes induced by PEDV. Tissue fixation was performed in 10% buffered formalin and ambedded in paraffin. Samples were stained with hematoxylin and eosin.
Results: 623 samples of intestines, faeces and rectal swabs from pigs with symptoms of epidemic diarrhea were analyzed during 2014-2015. 125 samples were positive for PEDV. Microscopic intestinal lesions such as vacuolation and necrosis of enterocytes were observed. Adhesion of the apical parts of villi was noted. It was also found infiltrations of lymphocytes and macrophages and hemorrhage in the lamina propria of the villi and submucosa. Such lesions could be caused by bacterial coinfection. Phylogenetic analyses of full genome of PEDV isolate detected in Ukraine showed the highest similarity with PEDV strains from USA and Mexico (GenBank accession no. KJ645707.1, KJ645700.1, KJ645708.1, KG643697.1). The nucleotide identity was 99,7%. The nucleotide identity with European and Asian PEDV strains was much lower.
Conclusion: Since the outbreak of PED in Ukraine we detected 125 positive samples of PEDV in pathological material with the use of in-house nested RT-PCR kit. Specific histopathological changes were characterized. Phylogenetic analyses showed high similarity with strains from USA and Mexico.

Disclosure of Interest: None Declared

Keywords: PEDv, phylogenetic analysis

Viral and Viral Diseases
PED

PO-PW1-042

Economic impact of PED outbreaks in naïve pig herds in the Netherlands

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Introduction: The impact of PED outbreaks in the United States had led to a high level of awareness in the Netherlands. A baseline study in the Netherlands, conducted in the second half of 2014, confirmed the naïve status of the Dutch pig industry for PEDv. After the study was completed the first outbreak of PED occurred in the Netherlands in November 2014. Until April 2015 a total of 51 outbreaks was confirmed. These outbreaks of PED were caused by low virulent strains of PEDv, strongly related to strains found in Germany and France and to US INDEL OH851, as was confirmed by sequence analysis. At that moment, no data on the economic impact of a PED outbreak under Dutch circumstances were available. Based on 11 cases we studied the production and economic impact of a PED outbreak in Dutch pig herds. This study was financed by the Dutch government and pig industry.
Materials and Methods: Eleven herds diagnosed with a PED outbreak were included: five finishing herds with 440 – 2300 finishers, one nursery unit with 1800 weaners and five sow herds with 300 – 1500 sows.
All herds were diagnosed from November 2014 until April 2015. The collected production data per finisher herd were mortality rate, average daily weight gain (ADWG), food conversion ratio (FCR), and delay in delivering to slaughter. For sow farms data on mortality of suckling and weaned piglets, growth rate and reproduction parameters were gathered.
Results: Overall the clinical picture and impact of an outbreak of PED varied between farms. In finishers the PEDv outbreak resulted in a reduced ADWG of 40 – 70 gram per day, the FCR worsened with 0,1 – 0,2, and loss of uniformity led to a delay in delivery to the slaughterhouse up to 21 days. In finishers there was hardly any extra mortality. On the sow farms economic damage consisted of mortality of suckling piglets during three weeks (ranging from 40 to 100%), decreased ADWG and increased mortality of weaners. In sows repeat breeders and abortions were recorded.
Conclusion: In finishing herds the average economic impact per finisher is estimated to be € 6. In sow herds the economic damage is mainly caused by mortality of piglets in the first week after farrowing during a rather short period of 3 weeks, and the poor quality of weaned piglets in the same period. We calculated the average economic impact per sow on commercial piglet producing farms in the Netherlands to be € 40 maximum.

Disclosure of Interest: None Declared

Keywords: economic, PED, the Netherlands

Viral and Viral Diseases
PED

PO-PW1-041

Monitoring and eradication of PED: experiences in eleven pig herds in the Netherlands

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Introduction: After the first signals from the US about devastating PEDv infections in 2013, Europe was extremely motivated to prevent this virus crossing the Atlantic. A baseline study in the Netherlands, conducted in the second half of 2014, showed the naïve status of the Dutch pig industry for PEDv. In November 2014, after the first case of PED was confirmed by GD Animal Health, a PED taskforce, in which the government and all involved organizations in the Dutch pig industry were represented, decided to approach this threat cooperatively.
GD veterinarians were asked to evaluate the effects of intervention strategies on 11 infected farms during 6 months after infection. This study was financed by the Dutch government and pig industry.
Materials and Methods: Five fattening herds, one nursery herd and five sow herds were selected after PEDv infection was confirmed by PCR. The route of introduction of each farm was established, to prevent new introductions. For control and eradication of PEDv, a tailor made advice, mainly based on biosecurity issues, was given. For the testing of feces an adapted commercial semi quantitative reverse transcriptase PEDv PCR was used.
Regular testing of pooled fecal samples was done to monitor the effect of interventions. In sow herds nursery piglets and replacement gilts were sampled, in fattening herds a random sampling in all age groups was performed. PEDv was considered to be successfully eradicated if three sampling rounds with at least 14 days interval, of thirty randomly taken individual fecal samples proved to be PCR negative.
Results: After the introduction of PEDv, the virus could be detected for 4-6 weeks at room level. At farm level the virus is much longer detectable due to transmission to new susceptible animals. However, we found that intervention via strict biosecurity can effectively prevent the transfer of PEDv to naive compartments in a fattening herd. Successful intervention after PEDv introduction on sow herds was achieved by infecting all sows simultaneously, followed by strict biosecurity. Three fattening -and three sow herds were declared “unsuspicious for PEDv” within 6 months after the diagnosis of PED was confirmed. The nursery herd was repopulated after double cleaning and disinfection. One fattening herd stopped farming.
Conclusion: The successful strategy to prevent the transmission of PEDv requires a tailor made approach, based on strict protocols and cooperation between motivated farmers and farm contacts. Regular monitoring of fecal samples is a useful tool to monitor the herd status and can motivate the farmer to keep up a high level of biosecurity until the virus is completely eradicated.

Disclosure of Interest: None Declared

Keywords: eradication, Netherlands, PEDv

Viral and Viral Diseases
PED

PO-PW1-049

Comparison of four different assays for the detection of porcine epidemic diarrhea virus (PEDV) in different stages of the infection in Italy.

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Introduction:
Porcine epidemic diarrhea virus (PEDV) and porcine deltacoronavirus (PDCoV) represent new threats to the swine industry. To aid in early detection of virus, monitor shedding, or differentiate viral species, real-time PCR has proven a useful diagnostic tool. The aim of this study was to measure the sensitivity of real-time PCR assays for the detection of PEDV and PDCoV on field samples at different times of the infection.

Materials and Methods:
A total of 20 samples were tested on four different real-time PCR assays, including three commercial assays and one in-house assay. The samples included feces from five animals at different times of the infection. The in-house assay was represented by two versions with different primers and probe concentrations of the real-time PCR assay developed by the University of Minnesota. One of the commercial assays was the recently launched RealPCR PEDV/PDCoV Multiplex RNA Test from IDEXX, which can be run on a modular platform with any other RealPCR test, with the two other ones being commercial multiplexes for PEDV and transmissible gastroenteritis virus (TGEV) and PEDV/TGEV/PDCoV. 200 µl of a 10 % feces homogenate was used as starting material and extracted using the Macherey-Nagel NucleoMag® VET 96 kit. The PCR reaction was prepared according to the manufacturer’s instructions and amplified using a Bio-Rad CFX96™ real-time PCR instrument.

Results:
All assays correctly identified the samples tested, with PEDV being positive in all samples. The TGEV and PDCoV targets were negative with all assays, as expected. The IDEXX RealPCR PEDV/PDCoV Multiplex RNA Test reached the lowest Ct-value in 16 samples out of the 20 samples tested, demonstrating the high sensitivity of the assay.

Conclusion:
These results demonstrate the high sensitivity and specificity of the IDEXX RealPCR PEDV/PDCoV Multiplex RNA Test. The RealPCR tests for swine enteric coronaviruses from IDEXX are configured as either single target, PEDV, PDCoV, and TGEV tests, or as a PEDV/PDCoV multiplex test. All configurations include an ISC for the detection of swine RNA as an internal control.

Disclosure of Interest: None Declared

Keywords: PCR, PEDV

Poster Abstracts

Viral and Viral Diseases
PED

PO-PW1-067

Porcine Epidemic Diarrhea (Ped) Virus, Genetic Analysis In Mexico

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Introduction:
Porcine Epidemic Diarrhea (PED), an alphacoronavirus infection of pigs, was first reported to the OIE by Mexico´s authorities on May 2014 based on results obtained by molecular techniques. The official report was limited to the pathogen identification with no sequencing information. With the objective of generating information regarding PED´s genotypes present in Mexico and to determine its geographical distribution, a sequence analysis was performed using samples obtained from different states of the country.
Materials and Methods:
The results of S1 region sequence from one-hundred-one PED viruses obtained between January 2014 and February 2015 were analyzed. Ninety-eight samples originated from six different states and three additional samples from 2013 were included. The sequence of the Illinois 63-2013 PED virus was used in order to compare our national sequences. Results were analyzed using Vector NTE ver. 11.0 for the generation of a dendogram and sequences with genetic distances of 1% or more were grouped as a new branch.
Results:
Our dendogram shows the presence of three main branches and more than forty clades as compared to the original type virus (Illinois 63- 2013).
Branch 1 contains sequences detected from the mid-western state of Jalisco; branch 2 from Jalisco and also from the north-western state of Sonora; and branch 3 from the state of Jalisco, the central states of Morelos, Mexico, Puebla and also from the state of Veracruz in the Gulf of Mexico.
Using the genetic distances of the analyzed sequences, we found that branch 3 has the widest genetic differences among all samples, with sequences with a variation of up to 1.5%.
Conclusion:
From one-hundred-one samples, three branches were identified. One sequence from Veracruz (2014) and one sequences from Jalisco (2015) showed a genetic distance of 1.5% like a possible representative of the virus evolution or continuous adaptation process. The rest of the samples remained within a close homology to the original virus Illinois 63-2013.

Disclosure of Interest: None Declared

Keywords: Genotype, Mexico, PED

Viral and Viral Diseases
PED

PO-PW1-040

Modeling the transboundary risk of feed ingredients contaminated with porcine epidemic diarrhea virus

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Introduction: This study describes a model developed to evaluate the transboundary risk of PEDV-contaminated swine feed ingredients and the effect of two mitigation strategies during a simulated transport event from China to the US.
Materials and Methods: Ingredients imported to the USA from China, including organic & conventional soybeans and meal, lysine hydrochloride, D-L methionine, tryptophan, Vitamins A, D & E, choline, carriers (rice hulls, corn cobs) and feed grade tetracycline, were inoculated with PEDV. Control ingredients, and treatments (ingredients plus a liquid antimicrobial (SalCURB, Kemin Industries (LA) or a 2% custom medium chain fatty acid blend (MCFA)) were tested. The model ran for 37 days, simulating transport of cargo from Beijing, China to Des Moines, IA, US from December 23, 2012 to January 28, 2013. To mimic conditions on land and sea, historical temperature and percent relative humidity (% RH) data were programmed into an environmental chamber which stored all containers. To evaluate PEDV viability over time, ingredients were organized into 1 of 4 batches of samples, each batch representing a specific segment of transport. Batch 1 (segment 1) simulated transport of contaminated ingredients from manufacturing plants in Beijing (day 1 post-contamination (PC)). Batch 2 (segments 1 and 2) simulated manufacturing and delivery to Shanghai, including time in Anquing terminal awaiting shipment (days 1-8 PC). Batch 3 (segments 1, 2 and 3) represented time in China, the crossing of the Pacific and entry to the US at the San Francisco, CA terminal (day 1-27 PC). Batch 4 (segments 1-4) represented the previous events, including transport to Des Moines, IA (days 1-37 PC).
Results: Across control (non-treated) ingredients, viable PEDV was detected in soybean meal (organic and conventional), Vitamin D, lysine hydrochloride and choline chloride. In contrast, viable PEDV was not detected in any samples treated with LA or MCFA.
Conclusion: These results demonstrate the ability of PEDV to survive in a subset of feed ingredients using a model simulating shipment from China to the US. This is proof of concept suggesting that contaminated feed ingredients could serve as transboundary risk factors for PEDV, along with the identification of effective mitigation options.

Disclosure of Interest: None Declared

Keywords: feed, PED, transboundary

Viral and Viral Diseases
PED

PO-PW1-075

RELATIONSHIP BETWEEN MATERNAL IMMUNE STATUS AND NEONATAL PROTECTION AGAINST PEDV INFECTION.

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Introduction: The objective of this experiment was to evaluate the impact of PEDV antibody (level and isotype) in colostrum and milk on clinical parameters in neonatal piglets inoculated with PEDV.
Materials and Methods: The study was conducted under the approval of the Iowa State University Office for Responsible Research (ISU #2-14-7736-S).
Two PEDV IFA-negative sows and 8 sows previously infected with PEDV ("principals") were acquired from a commercial farm at ~84-100 days of gestation and housed under experimental condition. Piglets derived from principals (n = 91) were orally inoculated with 1 x 10^3 $TCID_{50}$ PEDV (USA/IN/2013/19338E) at 2 days of age while piglets from control sows (n = 22) remained unchallenged. Thereafter, clinical signs and body weight were recorded daily through the termination of the experiment on day post-inoculation (DPI) 12.
Serum, colostrum, and milk were tested for PEDV IgG and IgA using a whole virus ELISA and for virus-neutralizing antibody using a fluorescent focus neutralization (FFN) assay. Feces were pooled by litter and tested by PEDV real-time, reverse transcriptase PCR (rRT-PCR). Data were analyzed for the effects of maternal PEDV antibody levels in colostrum and milk on piglet PEDV systemic antibody levels, fecal shedding, body temperature, weight gain, and mortality using logistic regression analysis.
Results: In control sow litters (n = 2):

- Neither clinical signs nor diarrhea were observed. Piglet mortality was 0 and 7.14% and piglet average daily gain (ADG) was 0.19 and 0.2 kg/day.

In litters from "principal" sows (n = 8):

- Diarrhea was observed in 27.3 to 100% of piglets.
- Mortality ranged from 0 to 40%.
- ADG ranged from 0.02 to 0.19 kg/day.
- Statistical analysis showed that colostrum and milk antibody titers significantly affected ($p < 0.05$) piglet percent weight change, PEDV shedding, and antibody levels on DPI 12.
- Piglet serum antibody levels (FFN, IgG, IgA) on DPI 0 significantly affected antibody responses on DPI 12 ($p < 0.0001$).

Conclusion: Combining the results of this experiment with previous work in the literature leads to the conclusion that both colostral and maternal antibodies in milk contribute to the protection of the neonatal pig against PEDV infection. Management of PEDV in commercial pig farms will depend on maintaining a sufficient level of PEDV immunity in sow herds.

Disclosure of Interest: None Declared

Keywords: Maternal immunity, Porcine epidemic diarrhea virus

Viral and Viral Diseases
PED

PO-PW1-078

DOES CIRCULATING ANTIBODY AFFECT THE COURSE OF PEDV INFECTION IN NEONATAL PIGLETS?

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Introduction: The work described herein used a "passive transfer model" to explore the impact of maternal (colostral) antibody on the course of PEDV replication and neonatal health. The question asked was, "*Does circulating antibody affect the course of PEDV infection in piglets?*"
Materials and Methods: The study was conducted under the approval of the Iowa State University Office for Responsible Research (ISU #2-14-7736-S).
Six PEDV IFA-negative sows were farrowed in research conditions. At 4-to-5 days of age, piglets (n = 62) were assigned to 1 of 6 treatments using a randomized block design such that all litters had 1 or 2 piglets with each treatment. Treatments consisted of intraperitoneally (IP) administration of 1 of 6 levels of concentrated PEDV antibody resulting in circulating FFN antibody titers of <1:8, 1:5.3, 1:6.1, 1:8, 1:17.1, and 1:32. 24 h later, all piglets were orally inoculated with PEDV (1 x 10^3 $TCID_{50}$ strain USA/IN/2013/19338E in 4 ml milk replacer) and then observed through DPI 14 or until humane euthanasia was necessary. Piglets remained on the dam throughout the observation period.
Data and samples collected on a daily basis included sow milk, piglet fecal samples, piglet clinical signs, body weight, and body temperature. Serum samples were collected from piglets at DPI -1, 0, and 14, or at the time of humane euthanasia. Fecal samples were tested by PEDV rRT-PCR. Piglet serum samples were tested for PEDV IgG and IgA by a whole virus-based ELISA and for PEDV FFN antibody. The effects of treatment on the outcomes measured were analyzed using appropriate statistical methods.
Results: • "Sow effect" on the course of PEDV infection in neonates was controlled by assigning all treatments to all litters (randomized block design). Analysis showed that "sow" had no effect on piglet survival.

- No difference among treated (PEDV antibody positive) and non-treated (PEDV antibody negative) pigs was detected in body weight, PEDV fecal shedding, or PEDV antibody responses.
- Compared to the antibody-negative control group, a difference was detected in time to death for treatment 5 ($p < 0.05$) and a trend toward a difference for treatment 6 ($p = 0.11$).
- Piglets that received PEDV antibody returned to normal body temperature sooner than control piglets, i.e., on DPIs 4, 5, 6, and 8 (p-value < 0.05).

Conclusion: Combining the results of this experiment with previous work in the literature leads to the conclusion that both colostral antibody (IgG) and maternal secretory IgA in milk contribute to the protection of the neonatal pig against PEDV infection.

Disclosure of Interest: None Declared

Keywords: Passive antibody, Porcine epidemic diarrhea virus

Poster Abstracts

Viral and Viral Diseases
PED

PO-PW1-057

PEDv elimination from two multiplication one-site farrow-to-finish herds

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Introduction: Methods for PEDv elimination on farrow-to-wean farms have been based on previous experiences with TGE and PRRS. However, methods for PEDv elimination on one-site farrow-to-finish farms have not been published and only anecdotal batch farrowing experiences exist. The objective of this case-report is to describe the methods by which PEDv was eliminated from two one-site multiplication farrow-to-finish farms.
Materials and Methods: Farm A (150 sows + 100 boars) and Farm B (350 sows) are 150 meters from each other and belong to the same multiplication flow. On April 3rd 2015 (calendar week 14), scouring suckling piglets were detected on farm A and a couple days later sows began vomiting. Suckling piglet fecal samples were sent for diagnostics confirming the presence of PEDv through RT-PCR. On April 8th a similar scenario was seen in farm B and samples sent to the laboratory were positive for PEDv.

An elimination program was put in place through partial depopulation together with load-close-homogenize intervention. Feedback was initiated on calendar week 15 and 16 for farm A and B respectively. Partial depopulation began on the second half of week 15 and continued for the following 2-3 weeks. Gestating sows with pregnancies of 50+ days, nursery and grower-finisher pigs were moved to an off-site farm. Once each building was empty, a strict sanitation protocol was initiated which included, dismantling farrowing crates, nursery pens, feeders in order to eliminate organic matter from the surface. This process was followed by power washing, detergent application and surface scrubbing. Buildings were allowed to dry and torched afterwards. Surfaces were then disinfected with Virkon S and allowed to dry. This process was repeated one more time before the buildings were inspected and then painted with lime. Monitoring was done at the University of Minnesota Diagnostic Laboratory by shipping fecal smears from boars (n=30) and suckling piglets (n=30) on FTA cards and testing through RT-PCR on pools of five.
Results: RT-PCR results from samples collected on week 18 and 22 yielded 3 and 11 positive and suspect pools, respectively. Samples collected on week 26, 28, 31 and 33 all yielded negative RT-PCR results. No diarrhea has been detected on suckling, nursery or grower-finisher pigs.
Conclusion: Elimination of PEDv from one-site farrow-to-finish farms is achievable through partial depopulation, load-close-homogenize and deep sanitation of buildings. Successful elimination of PEDv was due to the fact that close attention was given not only to duration of population shedding but also to environmental contamination.

Disclosure of Interest: None Declared

Keywords: Elimination, PED

Viral and Viral Diseases
PED

PO-PW1-047

PED elimination in a one-site farm using a novel approach

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Introduction: Owing to inexperience with Porcine Epidemic Diarrhea (PED) but building on experience with Transmissible Gastro-Enteritis (TGE), North American producers utilized various methods of whole-herd exposure and sanitation to eliminate PED virus from sow herds. Compared to continuous-farrow flows, elimination was easier in group-mating systems due to the inherent break in piglet ages/flow. Age-segregated flows with movement at weaning to off-site nurseries were easier than one-site farrow-finish flows due to lower viral exposure coming from sero-converting nurseries. This paper examines one elimination process on a farrow-finish site which reduced viral pressure by creating an artificial break in piglet production, achieving uniform whole-herd immunity, applying aggressive sanitation, and enforcing strict control of people and pig movement.
Materials and Methods: Acute PED appeared in a one-site farrow-finish 850 sow herd in an area of low swine density. The herd was considered "high-health," remaining PRRS/Myco negative for years. Anticipating the course of events, swift action was taken. All sows due to farrow in the next four weeks were sold and moved off-site in a matter of days. Healthy older piglets were weaned; susceptible younger piglets were humanely euthanized. Fostering between litters ceased. The entire production herd, including all replacement females was inoculated using dilute piglet diarrhea in oral/nasal spray. Emphasis on sanitation increased utilizing cold water, high pressure washing and disinfection with Synergize and bleach. Pathogen circulation within the facility was prevented by strict all-in/all-out pig movements and segregation of people and equipment.
Results: Clinical signs spread throughout the entire population, providing uniform whole-herd immunity. Light-weight pigs and those particularly stunted were removed from the site. Evidence of disease persisted 3-4 weeks at which time farrowing sows were able to provide lactogenic immunity. Following a testing protocol patterned by AASV, the facility was PED negative within seven months.
Conclusion: Prompt aggressive action was pivotal to the successful elimination. Removing late-term pregnant sows reduced the susceptible piglet population at a critical period and reduced the level of PED exposure in all farrowing rooms; thus reducing pressure on the sanitation process to prevent contamination between farrowing groups. Targeted exposure processes reduced the virus exposure level in gestation while providing uniform immunity. Strict adherence to “no fostering” prevented viral movement between litters. Altogether, the balance of exposure versus immunity tipped in favor of successful PED elimination.

Disclosure of Interest: J. Geiger Conflict with: PIC employee, J. W. Lyons Conflict with: PIC employee, J. P. Cano Conflict with: PIC employee, G. Shepherd: None Declared

Keywords: Elimination, PED

Viral and Viral Diseases
PED

PO-PW1-052

Epidemiological information of porcine epidemic diarrhea during the second epidemic year (2014 to 2015) in Miyazaki prefecture, Japan

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Introduction: Porcine epidemic diarrhea virus (PEDv) was first reported in Japan in the 1990s, and a PED live vaccine was approved in 1996 (Sueyoshi et al., 1995). Since then, only sporadic and relatively unimportant outbreaks were recorded. However, in 2013 and following the first PEDv case reported in Japan in seven years, detected in Okinawa prefecture in October, the virus rapidly spread across the country with 817 PEDv cases confirmed across 38 prefectures as of August 31, 2014.
Miyazaki prefecture is located on the southern island of Japan and is the major swine-producing area in Japan. In this prefecture, during the first year of the epidemic, PED was detected from December 13, 2013, to July 24, 2014. No more cases were detected until December 2014, when a new case epidemic wave started in Miyazaki prefecture. The objective of the study here was to investigate the epidemiological information of PED during the second epidemic year (2014 to 2015) in Miyazaki.
Materials and Methods: The present study was conducted in Miyazaki prefecture, Japan. Information on the location and characteristics of all swine farms in the prefecture was obtained from the prefecture database (including farm size, farm operation type, and basic information for livestock producers) in July 2015. The database included 511 pork producers. Between-farm distance and Kernel density was calculated using ArcGIS V10.2 (ESRI, Redlands, California, USA). Data on PED incidence in the region was obtained from Miyazaki Livestock Hygiene Service Center, and was available between December 2013 and July 2015. PED was first suspected based on the presence of clinical signs, and all cases suspected were confirmed by laboratory testing. Statistical analyses were performed in SAS V9.3 (SAS Institute Inc., Cary, NC, USA).
Results: During the first epidemic year (2013 to 2014), 81 out of 511 (15.8%) farms broke with PED. The first case was detected in middle December, and a sharp increase was observed in early February and middle March.
During the second epidemic year (2014 to 2015), 11 farms out of 511 (2.2%) farms broke with PED. Of the 11, 9 were new PED cases, whereas 2 were farms already positive in 2014. Of the total 11 cases, 7, 1 and 3 were farrow-to-finish farrow-to-wean, and wean-to-finish farms, respectively. The first case was detected in middle December, and the following six cases occurred until January 2015. The outbreak was mainly seen in the southwest part of the Miyazaki prefecture.
Conclusion: In conclusion, the number of PED occurrence during the second epidemic year was significantly lower than that during the first year. Further epidemiological data are needed to investigate risk factors associated with PED outbreaks.

Disclosure of Interest: None Declared

Keywords: Epidemiology, PED, rebreak

Viral and Viral Diseases
PED

PO-PW1-037

First detection of porcine epidemic diarrhea virus in Korean wild boars

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Introduction: Porcine epidemic diarrhea virus (PEDV) is characterized as highly contagious and severe digestive failure in domestic pigs. Wild boar (*Sus scrofa*) population is considered as reservoir of several viral pathogen such as CSFV, ASFV, ADV, PCV, HEV, and PRRSV. Nevertheless, there were no report on the prevalence and reservoir of PEDV in wild boar population. Therefore, we investigated the presence of the PEDV in the fecal samples of wild boar population during 2010-2011.
Materials and Methods: All of the 287 fecal samples of wild boar were obtained from hunters during 2010-2011. The samples were analyzed by RT-PCR and sequencing method with primer pairs which target partial spike genome. The nucleotide sequences were aligned by Clustal W program. The phylogenetic tree was constructed by MEGA 6 with reference sequences that were obtained from GenBank.
Results: The 28 samples were determined to be PEDV positive during 2010-2011. Positive rates of PEDV in wild boar was 9.9% (28/287). The samples were distributed throughout the mainland of South Korea. Especially, Gyeonggi and Gangwon provinces were the most prevalent regions of PEDV positive. The phylogenetic analysis showed that the genetic relation of PEDV of Korean wild boar was considerably adjacent with Chinese PEDV isolates (KP728470, KP162057, KJ158152, KC210146, JN601051, KR514323, JQ239432, KP403802, JN601045, JN547228 and KC886300), showing 95.5-100% identity.
Conclusion: This study is the first discovery of PEDV in wild boar population. The data suggest that the PEDVs in wild boar were broadly spread in Korean territory. This findings may provide the new perspective in control of PEDV in domestic pigs.

Disclosure of Interest: None Declared

Keywords: None

Poster Abstracts

Viral and Viral Diseases
PED

PO-PW1-062

Identification and characteristics analysis of the Porcine epidemic diarrhea virus (PEDV) Isolated In Shanghai

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Introduction:
Porcine epidemic diarrhea virus (PEDV) belonging to the Group I coronavirus is the major cause of lethal diarrhea disease in piglets. In recent years, there are many case reports of PEDV infection in pigs in China and caused large economic losses. To better control this disease, it's necessary to explore the epi demiological status and genomic characteristics of the new PEDV isolates.
Materials and Methods: Fifty five feces samples of piglets with diarrhea symptom in farms of Shanghai were collected and used for RT-
PCR detection. Then the postive samples were inoculated on Vero cells with trypsin for subculture. After detection of the cell cultures by RT -
PCR and indirect immunofluorescence assay (IFA) with monoclonal antibody (MAb) against PEDV, genomic characteristics of the isolates were analyzed u sing Lasergene software.
For further confirmation of the pathogen, six one -
day old piglets were divided randomly into three groups. Two groups were orally feed with 20ml concentrated virus 30 -
3 ($10^{5.4}$ $TCID_{50}$/0.1ml), W14 ($10^{3.4}$ $TCID_{50}$/0.1ml). The third group was used as control with DMEM inoculation. Clinical features especially the diarrhea sym ptom of the piglets were observed. Diarrhea feces and intestine contents of dead pigs were also collected and detected by RT-PCR.
Results: Two of fifty -
five samples were PEDV positive and were blindly passaged in Vero cells with different concentrations of trypsin. After several continuous passages, the t wo PEDV isolates in cell cultures were positive in RT - PCR and IFA with monoclonal antibody (MAb) against PEDV and were named 30 -
3and W14. Genomic comparison and phylogenetic analyses revealed that 30 -3 and W14 isolates share highly homology with the isolates of BJ -2012 -
1 and HLJ - 2012 respectively. There are 138 amino acids deletion in ORF3 of the isolates which were characterized of cell adapted strains.
Both of the two piglets inoculated with 30 -
3 isolate appeared diarrhea at 24h after inoculation and died at 4 dpi. Piglets inoculated with W14 showed clinical symptom of diarrhea and one pig died at 6 dpi. With post mortem examination, the small intestines were full of bleeding and gas, which was the typical characteristic of PEDV infection. The faeces and intestinal contents of the pigs inoculated with 30 -3 and W14 were PEDV positive.
Conclusion:
In conclusion, the two isolates of the PEDV were identified in vero cell successfully. And the phylogenetic analysis indicates the viruses were different form the classic CV777 and were similar with those isolated in China in recent years.

Disclosure of Interest: None Declared

Keywords: animal regression test, PEDV, virus isolation

Viral and Viral Diseases
PED

PO-PCO1-003

PIG TRANSPORTATION AS A KEY FOR DISSEMINATION AND MAINTENANCE OF PORCINE EPIDEMIC DIARRHEA VIRUS INFECTION IN COLOMBIA

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Introduction: Porcine epidemic diarrhea virus (PEDV) is a devastating enteric disease. In Colombia, PEDV was first reported in two departments in early 2014. The goal of this research was to evaluate PEDV presence on porcine transportation trucks from 14 different regions of Colombia as a contributing factor to the spread and maintenance of the disease in Colombia.
Materials and Methods: Environmental samples were collected from 520 trucks employed to transport live pigs destined for slaughterhouses. The samples were taken at two moments: upon arrival and after unloading from the abattoir (when trucks were disinfected). Samples were collected from June to October of 2014. The study included 32 abattoirs from 14 Colombian departments. Sample collection consisted of rubbing a PBS moistened pad (3M®) on the inner floor of the truck. RNA extraction was performed with the RNeasy® QIAGEN kit, first stand cDNA was synthetized by using the reverse transcription High Capacity cDNA synthesis Applied Biosystems® Kit and real time PCR was performed on a conserved region of the nucleocapside (N) gene, employing LightCycler® 480 probes Master-Roche mix. Primers and probes targeting the gene sequence were: forward, 5'GAATTCCCAAGGGCGAAAAT3', reverse 5'TTTTCGACAAATTCCGCATCT3', and probe FAM-CGTAGCAGCTTGCTTCGGACCCA-BHQ. All positive samples for gene N were re-checked for amplification of genes S (spike) and M. Samples were processed at the Laboratory of Animal Virology of the Universidad Nacional de Colombia, Bogotá D.C.
Results: In total, 483 of 520 trucks (92.8%) were contaminated upon arrival and 468/520 trucks remained contaminated after unloading. Only 7% of the trucks were not contaminated with PEDV upon arrival from the abbatoir. Samples from positive trucks showed the same cycle threshold values at arrival and departure. We found high viral loads of PEDV in departments where swine production is highest, such as Cundinamarca, Valle del Cauca, and Antioquia, with a Ct value rate of 32. At the beginning of the study (June and July), Ct values were lower compared to September and October (30.5 versus 36, respectively). We found PEDV in 8/14 departments (57%) and 17/32 slaughterhouses (53%). Finally, this study showed that 57% of the positive samples had Ct values between 30 and 35, which correspond to low viral loads (1x104 copies).
Conclusion: We confirmed the presence and spread of PEDV in different departments of Colombia. This study suggests that trucks can be an efficient source of dissemination of PEDV between farms and departments. The low viral load found in the majority of the samples shows that although the virus is circulating in Colombia, it is not showing fatal signs of illness.

Disclosure of Interest: None Declared

Keywords: enviromental samples, qPCR, trucks

Viral and Viral Diseases
PED

PO-PW1-081

Alpha and Delta Coronavirus Clinical and Diagnostic Observations of a Commercial Sow System

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Introduction: Intervention to a PED break includes initiating a multi-facet program focusing on a planned whole herd exposure, biosecurity and sanitation protocol with the goal of PED elimination. There is no literature following a group of individual sows and gilts over a years' time to measure fecal shedding, antibody levels and impact on performance. The objectives of this project were to evaluate: 1) Duration of shedding in individual sows 2) Individual sow antibody responses to exposure, and to 3) compare sample techniques.
Materials and Methods: Three 5,000 head, naive breed-to-wean sow farms in Eastern Colorado were followed over 365 days. All three sow farms broke with PED and Delta Coronavirus (SDCv) in March 2014. Once PED was confirmed each farm identified 30 random sows for serial sampling. These sows were stratified by location in gestation or lactation and parity (P0-P4+), resulting in 5 P0 from lactation and 5 P0 from gestation, similar stratification for P2-P3 and P4+. At each sampling, each individual sow had a rectal swab, oral fluid (OF) sample by individual rope and serum sample collected. This sampling protocol was repeated for six consecutive weeks, then every-other-week for six weeks, then another sampling 13 weeks later. The rectal swab and OF samples were tested for PEDv and SDCv PCR(BIVI HMC, Ames, IA). The serum was tested for PEDv Whole-Virus ELISA (ISU-VDL, Ames, IA.).
Results: All farms tested positive on PEDv ELISA and PEDv/SDCv PCR testing. SDCv positive animals were diagnosed soon after the PED planned exposure feedback. The percent positivity of both PEDv and SDCv, in both fecal swabs and OF showed a decreasing stair-step trend over time at each farm. The PEDv prevalence was over 75% positive the first two weeks with a steady decline thereafter. The antibody response at all farms was consistently high for 60+ days post-feedback. At approximately 80+ days post-feedback, there was a decrease (5-55%) in percent positivity at all farms which then increased to nearly 100% positive in subsequent weeks.
Conclusion: Oral fluid sampling of sows by individual ropes appeared to be the less valuable sample type for two reasons: 1) OF tests the individual pig and can include environmental contamination; 2) not all sows will chew the rope leading to inconsistent sampling. Fecal shedding of both PEDv and SDCv showed no consistent difference by age/parity. In general, the percent PEDv and SDCv PCR positivity of OF was higher and detected longer than rectal swabs. The overall duration of shedding of both PEDv and SDCv showed differences at each farm. The rise in PED ELISA percent positivity at day 80+ may be indicator of a recrudescence that occurred after the overall herd immunity waned.

Disclosure of Interest: None Declared

Keywords: Alpha, Coronavirus, Delta

Viral and Viral Diseases
PED

PO-PW1-043

Evaluating the immune response to serial administration of a conditionally licensed PEDv vaccine in naïve and previously exposed sows.

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Introduction:
Objectives
1) Determine the sow serum-neutralizing antibody titer (NABT) baseline status and response from a 2 dose blanket vaccination of Zoetis' conditionally licensed PEDv vaccine in both a naïve population, and a farm previously exposed to PEDv.
2) Determine the NABT level expressed in colostrum and milk after triple vaccination in these farms.
Materials and Methods: A historically naïve (Farm A) and a historically PEDv positive farm (Farm B), that had experienced an acute outbreak 6 months previously and that quickly achieved negative status were used in this study. On each farm, blood samples were collected from 30 animals prior to, and 3 weeks post-double herd blanket vaccination. In addition, colostrum (within 24 hours of farrowing) and milk samples (8-14 days post-farrowing) were collected from a subset of sows that were triple vaccinated at 8, 5 and 2 weeks pre-farrow. Serum samples from Farm B were collected at the time of colostrum collection. The fluorescent focus neutralization (FFN) assay was conducted on all samples at the South Dakota State University Veterinary Diagnostic Laboratory. Any sample with NABT ≥ 1:20 were considered positive.
Results:
Farm A:
Serum: Baseline status showed that all samples were negative. Post-double-blanket vaccination, 100% of all samples were positive with NABT ranging from 1:40-1:320.
Colostrum: All samples were positive with NABT ranging from 1:320–1:1280.
Milk: Two of 7 samples collected between 8-14 days of lactation were positive with relatively low NABT of 1:20.
Farm B:
Serum: Baseline status revealed positive NABT ranging from 1:320–1:2560 resulting from natural exposure. The NABT of most sows increased 2X after PEDv vaccination.
Colostrum: The NABT (range 1:1280->1:2560) were at least 2-4 times higher than in the serum (range 1:80-1:1280) of sows at the time of farrowing.
Milk: The NABT of samples collected at 4 days of lactation ranged from 1:40 to >1:2560. Additionally, 8/9 samples collected at 21 days of lactation remained positive.
Conclusion: Baseline sow serum NABT in the historically positive farm were higher, and showed higher serum NABT levels post-double vaccination than the naïve farm. In addition, these results show that NABT were detected in serum and colostrum following serial vaccination in both naïve herds and herds previously exposed to PEDv.

Disclosure of Interest: T. Wolff Conflict with: Zoetis, N. Baker: None Declared, J. Baker: None Declared

Keywords: PEDv

Poster Abstracts

Viral and Viral Diseases
PED

PO-PW1-063

EFFICACY STUDY OF PORCINE EPIDEMIC DIARRHEA VIRUS INACTIVATED VACCINE IN PREGNANT SOWS AGAINST AN EU PEDV ISOLATE

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Introduction: Porcine Epidemic Diarrhea virus (PEDV) was initially introduced in the United States in April 2013 and subsequently spread all over the country. In Europe, several outbreaks have been reported since 2014. Phylogenetic analysis of these new European isolates revealed that they cluster with the US INDEL variants associated with a milder disease presentation.
The conditionally licensed Porcine Epidemic Diarrhea Vaccine, Killed Virus, manufactured by Zoetis, is intended for pre-farrowing vaccination of sows and gilts against diarrheal disease in their neonatal pigs caused by PEDV. This vaccine was developed using a highly virulent American PEDV strain.
The objective of the study was to determine the immunogenicity and protection mediated by this vaccine when administered to pregnant sows and newborn piglets were challenged with an EU PEDV isolate.
Materials and Methods: The PEDV vaccine was administered intramuscularly to 5 sows and placebo to 3 sows in two 2 ml doses three weeks apart at 5 and 2 weeks prefarrowing. After farrowing, approximately at 4 days of age, all piglets were challenged with an EU PEDV variant, isolated from recent cases of diarrhea in neonatal pigs, which clustered with the PEDV INDEL variants. After challenge, piglets were evaluated for the presence of PEDV related clinical signs. Fecal swabs were taken to perform a PEDV-specific RT-qPCR. Three to four days post-challenge all piglets were euthanized and necropsied. Animal welfare statement: all in vivo work was conducted after ethical review, and in accordance with local, state, and national regulations.
Results: Antibodies were efficiently transferred to piglets as on the day of challenge, all piglets from placebo vaccinated sows had seroneutralizing titers ≤23 whereas all piglets from sows vaccinated with the PEDV inactivated vaccine had titers ≥ 23. There were no PEDV associated mortalities for piglets from PEDV vaccinated sows, whereas 23.8% of piglets in placebo group had PEDV associated mortalities attributed to challenge. After challenge, digestive disorders were reported in 90.5% of piglets from placebo vaccinated sows whereas only observed in 48.4% of piglets from PEDV vaccinated sows. Additionally, 66.7% of piglets from placebo vaccinated sows experienced loss of general physical condition and/or dehydration, whereas it was observed in only 3.2% of piglets from PEDV vaccinated sows.
Conclusion: The clinical data obtained confirm that the conditionally licensed Porcine Epidemic Diarrhea Vaccine, Killed Virus, manufactured by Zoetis, containing a US PEDV highly pathogenic isolate, is able to confer partial cross-protection to piglets born from vaccinated sows, against challenge with an heterologous EU PEDV isolate.

Disclosure of Interest: None Declared

Keywords: PEDv, vaccine efficacy

Viral and Viral Diseases
PED

PO-PW1-076

Autophagy Benefits the Replication of Porcine Epidemic Diarrhea Virus

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Introduction: The new porcine epidemic diarrhea (PED) outbreak has been documented in China since late 2010 and now with global distribution, resulting in enormous economic losses to swine industry. Autophagy is a highly conserved intracellular degradation process and be manipulated by some viruses for their benefits. Our previous proteomic data indicated that autophagy might participate in PEDV infection. However, the concrete role of autophagy is unknown. In the present study, we first detected the conversion of LC3-I to LC3-II, measured autophagic flux by monitoring p62/SQSTM1 degradation. Transmission electron microscope (TEM) was also used to observe autophagy induction. In addition, we demonstrated that whether the alteration of cellular autophagy by autophagy regulators and RNA interference affected PEDV replication.
Materials and Methods: The Vero cells were cultured in DMEM supplemented with 10% FBS to 80% confluence. Subsequently, cells were infected with PEDV (Accession no. KF761675) and incubated with serum-free DMEM containing 8 μg/mL trypsin. For pharmacological experiments, Vero cells were pretreated with optimal concentrations of drugs for 4 h prior to viral infection, and then infected with PEDV. For RNA interference, *Beclin* 1 was knocked down by transfected with *Beclin* 1 and scrambled siRNA with Lipofectamine 2000. Immunoblotting was performed to determine the conversion of endogenous LC3-I to LC3-II and p62/SQSTM1 degradation. The virus titers were determined by Reed–Muench method.
Results: To determine whether PEDV infection can induce autophagy, the conversion from LC3-I to LC3-II was monitored at 6 h, 18 h and 30 h post PEDV infection. It demonstrated that the conversion from LC3-I to LC3-II was significantly enhanced after PEDV infection. Meanwhile, the p62 degradation indicated that PEDV infection can increase the level of autophagic flux in infected Vero cells. TEM observation showed that autophagosome-like vesicles were significantly increased in Vero cells post-infection compared with mock-infected. When treatment with autophagy inducer rapamycin, the virus yield was increased, while treatment with the inhibitor 3-MA, the virus yield was reduced. Knockdown of the essential endogenous Beclin 1 also reduced PEDV infection.

Conclusion: In the present study, we first demonstrated that PEDV infection can induce autophagy, and then assessed the impact of autophagy on viral replication. We postulate that autophagy might be a potential mechanism that PEDV manipulated to benefit replication.
Acknowledgment
This work was supported by grants from the China Agricultural Research System (CARS-36).

Disclosure of Interest: None Declared

Keywords: autophagy, porcine epidemic diarrhea virus (PEDV)

Viral and Viral Diseases
PED

PO-PW1-044

THE PREVALENCE AND MOLECULAR EPIDEMIOLOGY OF PORCINE EPIDEMIC DIARRHEA VIRUS IN TAIWAN

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Introduction: Porcine epidemic diarrhea (PED) is an acute and highly contagious enteric viral disease in swine. In 2013, the disease has emerged and re-emerged in American and Asian countries, and caused severe economic loss in affected farms. The clinical signs of PED were observed in infected pig by vomiting, severe watery diarrhea, dehydration, and high morbidity and mortality, especially for suckling pigs. Since December 2013, the epidemic situation of diarrhea in the piglets had been remarkably deteriorating in Taiwan. During January 20, 2014 to December 30, 2015, a total of 333 diarrhea cases from 130 farms in 13 counties were submitted to the Animal Health Research Institute. The affected farm was observed severe diarrhea and vomiting in pigs of all ages. The objective of the present work was to survey the prevalence and molecular epidemiology of PED outbreaks in Taiwan.
Materials and Methods: The enteric samples were examined by reverse transcription polymerase chain reaction (RT-PCR) for differential diagnosis, including transmissible gastroenteritis virus (TGEV), porcine epidemic diarrhea virus (PEDV), and porcine group A rotavirus (GARV).Isolation of PEDV from field specimens was conducted in Vero cells. The supernatant of cultured Vero cell was examined by electron microscopy and RT-PCR test.The S gene of isolated PEDV were sequenced and compared with that of published PEDV sequences and were aligned by MUSCLE using MEGA 5.2 software. The phylogenetic tree of PEDV S gene was constructed by maximum-likelihood method.
Results: Among 333 specimens, 79 were positive for PEDV by RT-PCR. The prevalence of PED in farms was 40% (52/130) from 2014 to 2015. According to S gene sequences, the novel isolated PEDV Taiwan strains were clustered in group 2, together with strains PEDV/USA/2013, PEDV/ China/2012, and PEDV/Korea/2014, whereas the classical CV777 strain and historical Taiwan strain were rooted in group 1. The novel strains isolated in Taiwan were classified into 2 type strains (novel and variant novel strains) by S1 gene deletion, the variant novel strain has 205aa deletion between 23-227 positions in S1 gene. Among 52 novel PEDV outbreak farms, only 6 cases/farms were affected by the variant and novel PEDV simultaneously.
Conclusion: Since December 2013, the severe re-emergence of PED in Taiwan was caused by variant and novel strain. Compared with the historical PEDV, the novel PEDV strain was highly virulent and transmitted rapidly that caused high mobility and mortality in sucking pigs. The origin of the novel PEDV is still under investigation, and further studies on the virulence and antigenic characterization between variant and novel PEDV are required for developing a control strategy on PED.

Disclosure of Interest: None Declared

Keywords: MOLECULAR EPIDEMIOLOGY, PORCINE EPIDEMIC DIARRHEA , S1 GENE DELETION

Viral and Viral Diseases
PRRS

PO-PW1-094

Molecular identification and phylogenetic analysis of NSP2 and ORF5 genes of PRRSV in China 2012-2015

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Introduction: Porcine reproductive and respiratory syndrome (PRRS), caused by PRRS virus (PRRSV), is a major threat to the swine industry worldwide. The genome of PRRSV is characteristic of its extensive genetic variation, resulting in the complexity of not only genotype but strain diversity. In China, the distribution and ranks of the different PRRSV strains remain unknown in the swine herds although classical, high pathogenic (HP-) and novel variant PRRSVs co-exist. This study revealed the temporal distribution and ranks of PRRSV strains in Henan province of China during 2012-2015 based on the molecular epidemiology and evolutionary characteristics of PRRSV.
Materials and Methods: Initially, total 468 tissue samples were collected from diseased pigs at different ages from 165 herds in Henan province, China, during 2012-2015.Full-length ORF5 gene and truncated NSP2 gene of PRRSV were amplified by RT-PCR with sero-/geno-type-specific primers and PCR products were sequenced and analyzed by DNAstar, and phylogenetic trees were constructed by MEGA5.
Results: Of 468 samples, 141 (30.1%) were positive for PRRSV. In 2012, 80% of samples detected were HP-PRRSV positive, and 10% of samples were NADC30-Like positive. Notably, however, NADC30-Like positive samples increased to 78.2% (68/87) in 2015, showing a much higher incidence than that in 2012. In contrast, the incidence of HP-PRRSV positive samples decreased from 80% in 2012 to 18.4% in 2015.Meanwhile 65 NSP2 and 79 ORF5 sequences were obtained from a total of 79 filed strains. Phylogenetic trees revealed that all 79 strains belonged to genotype 2 of PRRSV and were clustered into Subgroup 2 represented by JXA1 (HP-PRRSV) and Subgroup 4 represented by NADC30, respectively, indicating that NADC30-Like and HP-PRRSV strains were the dominant strains in Henan province.
Conclusion: Since the first report in China, PRRS has been existed and evolved for 20 years and caused severe economic losses. Since 2011,HP-PRRSV-derived live vaccines have been commercially launched for compulsory immunization against PRRS in China, and then such vaccine-related PRRS cases increased during 2012-2014 followed by a decrease in 2015 with a less use of the vaccine. In addition, PRRSV strain NADC30 was isolated in U.S.A in 2008, and then the clinical cases remarkably increased over the following years. In China, a historical import amount of sows from U.S.A reached the peak in 2012 and from then on NADC30-Like strains have been increasingly identified from the herds following the distribution of the sows and their offspring. This study warns that people should pay more attention to the use of HP-PRRSV live vaccines and to the quarantine when importing animals.

Disclosure of Interest: None Declared

Keywords: NADC30-Like strain, NSP2, ORF5

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-118

Recombination analysis of full-length genome of PRRSV strains isolated in China 2013-2015

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Introduction: Porcine reproductive and respiratory syndrome (PRRS), caused by PRRS virus (PRRSV), still engenders immense economic losses to the swine industry worldwide. The co-existence of multi–type stains, rapid evolutionary rate and possible recombination of PRRSV lead to increased genetic diversity. Here we report the important recombination of 3 Chinese PRRSV strains collected during 2013-2015.
Materials and Methods: Full-length genome of four PRRSV isolates from swine herds in central China from 2013 to 2015 named as HENAN-XINX,HENAN-HEB,HENZMD-9 and HENZK-1, respectively, were obtained by RT-PCR and analyzed together with 24 reference strains including serotypes 1(EU) and 2(NA) strains by ClustalW and RDP3.44 softwares to retrieve possible recombination event. The authenticity of the event was verified by sequence analyzing of each part of the recombination.
Results: Compared with references, three (HENAN-XINX, HENAN-HEB and HENZMD9) of 4 isolates were identified as genome recombination strains. All recombination events occurred among NA not EU serotype strains. Acting as a backbone of all 3 variants isolated in this study, strain NADC30 recombined with NSP2-5 genes (4188-5997 nt) of a classical PRRSV strain S1 resulted in HENAN-XINX, recombined with NSP2 gene (1352-2135 nt) of a HP-PRRSV strain 09JS resulted in HENAN-HEB , whereas recombined with the genes of 2 HP-PRRSV strains, 5' UTR and NSP1-2 genes (1-1499 nt) of strain GX1002 and NSP2-9 genes (4093-7640 nt) of strain JXA1-P45, resulted in HENZMD9. However, there was no evidence of recombination in HP-PRRSV strain HENZK-1.
Conclusion: As a common pattern of genetic variation for RNA viruses, recombination is important to virus evolutionary process. PRRSV recombination was firstly reported in 1997. Since 1996, all classic, HP- and NADC30-like PRRSV strains belonging to NA serotype have been reported in China successively, and recently EU serotype strain was also reported. Thus, PRRSV strains developed diversified, and the co-existence of different strains in a pig farm enhances the risk of PRRSV recombination, which is one of the important factors making the PRRS control difficult. This study indicated that a) all three kinds of NA serotype PRRSV(classical, HP- and NADC30-like) co-existed in the herds in China. b) Three isolates were identified as variants derived from the recombination between Chinese strains (S1**,** 09JS , GX1002 and JXA1-P45)and USA strain NADC30 and c)As a backbone virus strain NADC30 suggested a strong ability to receive the foreign genes from the other NA serotype PRRSVs, but the mechanism remains unknown.The study on the pathogenicity of the isolates is under conduction.

Disclosure of Interest: None Declared

Keywords: NADC30-Like strain, PRRSV, recombination

Viral and Viral Diseases
PRRS

PO-PCO1-010

Impact of oral fluid handling on sensitivity of real time PCR to detect PRRSV

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Introduction: Oral fluid is convenient sample for monitoring of PRRSV in pig herds. However, due to its nature, it is recommended to maintain cold chain during transport and storage. The aim of the study was to evaluate the impact of storage conditions of oral fluid on sensitivity of real time PCR to detect PRRSV.
Materials and Methods: Oral fluid was obtained from a PRRSV-positive farm, transported to the laboratory, and stored at -20°C. The sample was extracted with RNA Mini Kit (Qiagen) and tested by Real-time PCR with EZ-PRRSV MPX 4.0 reagents (Tetracore Inc.). Obtained Ct was 28.00. The sample was diluted ten-fold in PRRSV negative oral fluid, from 10^{-1} to 10^{-3}. The undiluted sample and each dilution was stored at room temperature (20-25°C) without additives, or after adding equal volumes (1:1) of 0.1% chlorhexidine, 0.01% chlorhexidine or PBS, that served as preservatives. At days 0, 2, 4 and 6 one tube from every treatment and dilution series was placed in -20°C. Next, RNA was extracted from all samples, and Real-time PCR was performed as above. Each extracted RNA was run with PCR in five repeats.
Results: As expected the Real Time PCR Ct values increased from day 0 to day 6. After 6 days of undiluted oral fluid storage at room temperature the best results were obtained for samples preserved with 0.1% or 0.01% chlorhexidine. The mean Ct values were 30.25 (SD 0.73) and 30.88 (SD 0.36), respectively, compared to Ct 32.94 (SD 0.3) for samples mixed with PBS, or Ct 34.27 (SD 0.45) for untreated samples. The increase of mean Ct values between day 0 and 6 was 6.27, 0.73, 1.87 and 4,36 for native oral fluid and preserved with 0.1% chlorhexidine, 0.01% chlorhexidine and PBS, respectively. All oral fluid samples diluted 10^{-1} were positive at day 6 only when preserved with either chlorhexidine or PBS. PRRSV was detected in preserved oral fluid also diluted 10^{-2}, in 3 or 2 out 5 samples, mixed with 0.1 and 0.01% chlorhexidine, respectively. The mean Ct values of positive samples at day 6 were 35.48 (SD 0.58) and 37.82 (SD 0.93).
Conclusion: Oral fluid may contain many factors that compromise PCR sensitivity. Also, in field conditions it can be exposed for increased temperatures that can also decrease PCR sensitivity. In the experimental set up of this study, the sensitivity of Real Time PCR performed in unpreserved oral fluid dropped more than 100 fold between day 0 and 6 (the rise of the mean Ct value by 6,27). On the other hand, preserving oral fluid samples with chlorhexidine fully protected genetic material of PRRSV and the sensitivity of PCR was not decreased even after 6 days of storage of the samples at room temperature.

Disclosure of Interest: None Declared

Keywords: oral fluid, PRRSV, stability

Viral and Viral Diseases
PRRS

PO-PW1-196

Comparative analyses of PBMC transcriptome profiles between German Landrace and Pietrain pigs following PRRSV vaccination

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) is a devastating viral disease affecting swine industry worldwide. Innate immune response to PRRS virus (PRRSV) infection varies among pig breeds. Elucidating the role of host genetics in the variation of PRRSV vaccine responsiveness may lead to characterize the host immunocompetence and thereby resistance to PRRS. Therefore, the current study aimed to investigate the breed difference in innate immune response to PRRSV vaccination between German Landrace (DL) and Pietrain (Pi) pigs.
Materials and Methods: We employed a total of 12 Affymetrix GeneChip porcine gene 1.0 ST array for transcriptome profiling of peripheral blood mononuclear cells (PBMCs) collected before (0h) and 24h after PRRSV vaccination from three female piglets of four weeks old from of both DL and Pi breed. Normalization and statistical analyses of microarray data was performed with the 'oligo' and 'limma' Bioconductor package in R software. The gene ontology and pathway analysis was performed in the InnateDB pathway analysis tool.
Results: With FDR<0.01 and log2 fold change 1.5 as cutoff criteria, 4269 transcripts were found to be differentially expressed in PBMCs among four contrast pairs (*i.e.* DL-24h *vs* DL-0h, Pi-24h *vs* Pi-0h, DL-0h *vs* Pi-0h and DL-24h *vs* Pi-24h) tested. The number of vaccine induced differentially expressed genes (DEG) was much higher (DL-0h *vs* DL-24h, DEG=2459) in Landrace pigs than that of Pietrain pigs (Pi-24h *vs* Pi-0h, DEG=291). Before vaccination, 3255 genes showed differential expression between DL and Pi (DL-0h *vs* Pi-0h) which indicated the genetic variation between two breeds. After 24 h of PRRSV vaccination, 1046 genes were over expressed in Landrace pig compared to Pietrain (DL-24h *vs* Pi-24h) which indicated the breed differences in vaccine responsiveness as well. The top ten biological pathways significantly affected by genes differentially expressed in four contrast pairs tested includes *Cytokine signaling in immune system, Pathway in cancer, GPCR signaling, JAK STAT signaling, Interferon signaling, Autoimmune thyroid disease, Natural killer cell mediated cytotoxicity, Hepatitis C, Toll-like receptor signaling pathway* and *RIG-like receptor signaling pathway.* Majority of the pathways are linked to immune response function.
Conclusion: These findings provided an insight into the gene expression changes associated with innate immune response to PRRSV vaccination between German Landrace and Pietrain pigs. This study revealed that German Landrace pigs showed greater transcriptional responses to PRRSV vaccine in peripheral blood compared to the Pietrain pigs.

Disclosure of Interest: None Declared

Keywords: PRRSV vaccine, innate immunity, microarray

Viral and Viral Diseases
PRRS

PO-PW1-150

Molecular characterization of genotype 1 Porcine reproductive and respiratory syndrome virus in Korea : ORF 4-6 based analysis

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Introduction: Genotype 1 porcine reproductive and respiratory syndrome virus (European type PRRSV) has been rampant and resulted in extensive economic loss throughout Korea since the virus was first detected in 2005. Nevertheless, molecular analysis of EU genotype PRRSV has been limited to ORF5 and/or ORF7. Here, we determined the positive rates of EU PRRSV in Korea and molecular analysis of ORF4-6 sequences of Korean genotype 1 PRRSV.
Materials and Methods: A total of 669 serum samples from 27 pig farms during 2012 and one isolates of PRRSV during 2015 were analyzed by PCR amplification and sequencing. All of the ORF4-6 nucleotide and amino acid sequences were aligned with reference sequences using Clustal W. The phylogenetic construction was conducted in MEGA 6 with neighbor joining method. The prediction of N-glycosylation sites and hydropathy plots were implemented by NetNGlyc 1.0 Server and ProtScale on the Web, respectively.
Results: The 64 samples were detected as type 1 PRRSV positive, and 10 representative ORF4-6 sequences were analyzed among positive samples. The positive rates of EU PRRSV in Korea was 9.57% (64/669). The sequences covering ORF 4 to 6 presented to have 87.2-89.4 % sequence homology with reference strains (Lelystad). In the comparison with Lelystad strains in individual ORF of Korean strains, the ORF4 was the highest variable region among the ORF4-6 sequence. (ORF4: 82.7-87.3, ORF5: 87.1-89.7, and ORF6: 89-92.1%) Phylogenetic analysis of Korean strains showed that the Korean strains form independent clusters. In the amino acid sequence analysis of ORF4, variation of amino acid sequences was focused on the site of the 49 to 72 a.a. position, which was regarded as epitopes. The hydropathy plot corresponding to epitopes were highly variable in GP4. N-glycosylation sites of GP4 were three sites in N88, N124, and N134. In the ORF5 analysis of Korean strains, the amino acid variations were found in the site of 56-63 and 100-106 a.a. position. Potential N-glycosylation sites of Korean strains were divided into two forms (5 strains: N37, N46, and N53; 5strains: N37 and N53). The hypervariable regions in hydropathy plots of GP5 were focused on the sites of 33-67 and 89-109 amino acid regions.
Conclusion: Current study suggest that the genotype 1 PRRSV in Korea have substantially diverse properties in genetic and molecular level. Moreover, this study suggest that independent evolutionary properties of Korean EU PRRSV. Therefore, this study may be providing an evolutionary indicator for the analysis of Korean and global genotype 1 PRRSV.

Disclosure of Interest: None Declared

Keywords: None

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-156

'Piglet Monitoring' in Northern Belgium: phylogenetic and geographic distribution of PRRSv isolates

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Introduction: In Flanders, the Porcine Reproductive and Respiratory Syndrome virus (PRRSv) and Porcine Circovirus 2 (PCV2) are endemic on most herds and recognised as two major pathogens with a significant economic impact in the domestic pig. In order to monitor the infection status of a herd concerning PRRSv and PCV2 a voluntary program started in February 2015 with financial support of the Belgian Fund for Animal Health (For more details, refer to the companion abstract 'Piglet Monitoring' in Northern Belgium: a tool for veterinarians and farmers to control PRRSv and PCV2). Following-up the circulating PRRSv strains can be used by veterinarians as an interesting tool for evaluating and adapting the advices to the farmers regarding PRRSv surveillance, prevention and control strategies.
Materials and Methods: A phylogeny study based on partial sequences was performed on PRRSv positive samples collected in herds during two sampling campains. Up to now, 114 individual sequences in the ORF5 region were obtained [Stadejek et al., 2008] and aligned with others available in Genbank, including the European and American vaccine strains and Belgian isolates from 1994 to 2013 for phylogenetic study based on the maximum likelihood method.
Results: Among the 114 sequences, 102 were grouped in the European genotype and specifically in subtype 1.1. They shared identities ranging from 76 to 89% with Lelystad, the subtype reference strain. Two additional isolates were identified as European vaccine strains. In the subtype 2 (North-American) the 10 isolates were identical (96-99%) to the American vaccine strain.
Sample collection points were distributed in the four provinces from Flanders: Oost-Vlaanderen (n=13), West-Vaanderen (n=43), Antwerpen (n=50) and Limburg (n=8). No strict clustering could be observed in relation with the geographical distribution by province. However, one phylogenetic cluster seemed largely present in the four provinces when some others were predominantly distributed in one or two provinces. A more local analysis indicated that up to seven different strains may be circulating on the same municipality or 2 to 3 different strains in the same herd.
Conclusion: The screening that has started in Flanders one year ago has already demonstrated the diversity of the circulating PRRSv isolates in pig herds. The field isolates were all grouped in the European group and particularly in subtype 1.1 clustering with Lelystad strain. Up to now there is no evidence of the presence of field isolate from other European subtypes or from genotype 2. The signification of both geographically dispersed and localised clusters should be further investigated.

Disclosure of Interest: None Declared

Keywords: phylogenetic analysis, Porcine Reproductive and Respiratory Syndrome virus

Viral and Viral Diseases
PRRS

PO-PW1-121

Cross-sectional study one year after an acute PRRS outbreak

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Introduction: PRRSV outbreaks have recently been described in several boar studs in Germany, though only few farrowing farms have observed clinical signs. In the present study, 14 farrowing farms with different vaccination schemes were included who reported severe clinical infection after insemination with PRRSV contaminated semen. For these 14 farms, the PRRSV infection status was recorded one year after the initial outbreak, taking into account their vaccination schedules.
Materials and Methods: Fourteen farms who reported a field infection with PRRSV one year prior to our study were included. All farms carried out a PRRSV vaccination with the vaccine Porcilis® PRRS i.m.(MSD Animal Health, Intervet Germany). To evaluate the PRRSV status one year after the outbreak, on each farm blood samples were taken of 30 suckling piglets, 10 pigs at the middle of the nursery period and 10 pigs at the end of the nursery period. Samples which tested PRRSV-positive by PCR were further sequenced to distinguish between vaccination and field strains.
Results: One year after the initial outbreak, no farm showed any clinical signs of an infection with PRRSV. Only 8% (57 from 710) of the collected samples from ten farms were tested positive for PRRSV genotype I. Of these samples, 5.6% belonged to suckling piglets, 6.9% to pigs at the middle of the nursery period and 17.7% to pigs at the end of the nursery period. In farms with sow vaccination only no infection with PRRSV genotype I could be determined in the suckling piglets. In contrast, significantly ($p = 0.012$) more positive samples of suckling piglets were found in sow and piglet vaccinating farms (9.0%). Considering phylogenetic analysis, the PRRSV field strain that was responsible for the outbreak at the end of 2013 was re-isolated in five farms (two sow vaccinating farms and three sow and piglet vaccinating farms). In all of these farms, the field strain was detected in the nursery pigs (middle of the nursery period: 1/5; end of the nursery period: 5/5). In addition, the field strain was re-isolated in suckling piglets in two of the farms which vaccinated the sows and the piglets. In contrast, the PRRSV vaccine strain was only isolated in sow and piglet vaccinating farms (suckling piglets: 3/6, middle of the nursery period: 4/6, end of the nursery period: 1/6).
Conclusion: In five farms the PRRSV field strain could be identified one year after the initial outbreak. Most positive samples were detected in the pigs at the end of the nursery period. In farms which were still infected with the initial field strain the clinical situation could be controlled by vaccination of the piglets.

Disclosure of Interest: None Declared

Keywords: PRRSV infection, PCR, PRRSV-persistence

Viral and Viral Diseases
PRRS

PO-PW1-198

Screening of Seventeen Asian Medicinal Plants for Antiviral Activity against Field PRRSV Strain in Taiwan

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) is characterized by reproductive failure of sows and respiratory problems of nursery and growing pigs. Present management strategies mainly focus on the prevention of infection using vaccination but are not sufficient to eradicate the virus and provide complete immunity. Therefore, in searching for agents that may prove clinically effective against PRRSV infection, seventeen Asian medicinal plant extracts were screened for their antiviral activity against field PRRSV strain in MARC-145 cells.
Materials and Methods: An immunofluorescence assay (IFA) was detected viral replication, MTT assay was employed for analysis of cytotoxicity test and quantitative polymerase chain reaction (qPCR) were conducted to detected the effect of these extract on viral loads.
Results: Based on the results obtained from the cell viability tests, the highest concentration showing less than 10% cytotoxicity in MARC-145 cells was chosen for each for the plant extracts. The antiviral activity assay indicated that *Nepeta cataria, Cinnamomum cassia* and *Perilla fructescence* extracts had a significant reducing the PRRSV load *in vitro*.
Conclusion: In the future, we will be investigating further the antiviral mechanisms of this plant extract on PRRSV infection and applying to clinical trials on a swine farm.

Disclosure of Interest: None Declared

Keywords: Asian herb extracts, field PRRSV strain, qPCR

Viral and Viral Diseases
PRRS

PO-PW1-167

Post-vaccination safety of PrimePac PRRS™ in 2-week-old piglets and lactating sows

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Introduction: PrimePac PRRS is a modified live PRRSv Type II vaccine. Safety of Prime Pac PRRS was demonstrated in two overdose safety studies in both lactating sows and 2-wk-old piglets. A vaccine dissemination study was performed in 2-wk-old piglets.
Materials and Methods: Overdose safety in 2-wk-old piglets (Study 1): 6 piglets each were vaccinated intradermally with IDAL device (ID, 0.2 mL) with an overdose of one of two Prime Pac PRRS batches (B1; B2, produced by MSD AH, The Netherlands). For 14 days, piglets were observed for abnormal local or systemic post-vaccination reactions. In addition, rectal temperatures were measured prior to, 4 hrs after and daily for 4 days post-vaccination.
Dissemination and shedding in 2-wk-old piglets (Study 2): 2 groups of 21 2 wk old piglets each were vaccinated with Prime Pac PRRS either intramuscularly (G1; IM, 1mL) or ID (G2; 0.2mL). At 5, 11, 15, 21, 28, 35 and 42 days after vaccination, 3 piglets per group were sacrificed and samples were tested for PRRSv via virus isolation: blood, urine, faeces, nasal secretion, tonsils and lung lavage.
Overdose safety (Study 3): Lactating sows were vaccinated with a 10x overdose via IM or ID routes. Progeny were assigned to 4 groups: 1) 10x overdose IM and ID; 2) one dose IM and ID, 4 weeks later with a repeated dose; 3) single dose IM; 4) single dose ID. One group of lactating sows and two groups of piglets served as non-vaccinated controls. Post-vaccination observations included: rectal temperatures, clinical signs, injection site reactivity and weight gain of progeny.
Results: Study 1 - Abnormal local or systemic reactions were not observed in any of the piglets. At 4 hours and 2 days post-vaccination, the average body temperature was slightly elevated: respectively 0.3°C and 0.4°C for batch B1, and 0.1°C and 0.3°C for batch B2.
Study 2 - Regardless of vaccination route and at various time points, PRRSv was detected in 23.8% of serum samples, 38.1% of lung lavage samples (positive up to 42 days) and 28.6% of tonsil samples. Virus was not found in nasal secretions, urine and faeces at any time point.
Study 3 - No post-vaccination clinical signs were found in lactating sows or piglets and increased rectal temperature was of no biological relevance as was within the normal range. Lactating sow vaccination did not impact weight gain of progeny. Overdose vaccination or vaccination with single or repeated dose of piglets from vaccinated sows had no negative effect on weight gain. Small transient local reactions were found at IM and ID injection sites in lactating sows and piglets.
Conclusion: Prime Pac PRRS is safe for ID or IM vaccination of lactating sows and 2 week old piglets.

Disclosure of Interest: None Declared

Keywords: PRRS MLV vaccine, safety

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-180

Onset of immunity is four weeks following intradermal or intramuscular vaccination of 2 week old piglets with PrimePac PRRS™

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Introduction: The objective of this study was to evaluate the onset of immunity of PrimePac PRRS, a modified live PRRSv Type II vaccine, in piglets vaccinated intramuscularly (IM) or intradermally (ID) with IDAL, followed by challenge with PRRS Type II strain VR2332 4 weeks later.
Materials and Methods: Two-week old piglets were vaccinated with one dose PrimePac PRRS vaccine (produced by MSD-AH, The Netherlands) dissolved in Diluvac Forte. Vaccinations were done in the neck with either 1 ml IM (G1; n=11) or 0.2 ml ID (G2; n=10). Piglets in a third group (G3, n=10) were non-vaccinated challenge controls. Piglets were challenged intranasally 4 wks after vaccination with PRRSV Type II strain VR-2332. Ten days after challenge half of the animals in each group were sacrificed for post-mortem investigation of lung lesions with a weighted lesion score, and remaining pigs were examined 28 days after challenge. Other variables measured included PRRSv serology (IDEXX PRRS X3; S/P ratio); viremia and nasal shedding measured on porcine alveolar macrophages (AUC – area under curve); clinical signs; rectal temperature and weight gain between 10 and 27 days post-challenge.
Results: After vaccination or challenge, no PRRSV related clinical signs were observed in any of the piglets. Post-challenge rectal temperature was significantly lower in G1 (-0.7°C) and G2 (-0.7°C) than in G3.
Weight gain between Day 10 and 27 was significantly higher in vaccinates than control pigs (G1 +0.245 kg; G2 +0.171 kg).
At challenge, G1 and G2 pigs were PRRSv seropositive while G3 pigs were seronegative. By 10 dpc, all G1, G2 and G3 pigs seroconverted with the highest S/P ratios in G1 and G2. Post-challenge PRRSv viremia was significantly lower ($p<0.05$) in G1 (AUC: 17.4) and G2 (AUC: 15.2) compared to G3 (AUC: 22.2) pigs during the first 10 days of challenge and PRRSv could not be isolated from nasal fluids in any of the animals. The percentage of all collected serum samples that were found virus positive was 68% (G1), 67% G2) and 79% (G3).
Lung lesion scores were low, ranging from 0.99±2.15 to 4.97±3.34 for all three groups and tended to be lower in G1 and G2 than G3. These differences were only significantly different between G1 and G3. Although the odds for lower lung lesions on Day 28 were 13.7 and 6.07 times higher in G1 and G2 pigs, respectively, than in G3.
Conclusion: Two week old piglets vaccinated intradermally or intramuscularly were protected from a virulent PRRSv challenge 4 weeks later as demonstrated by the absence of increased rectal temperature, reduced level of viremia, weight loss and lung lesions.

Disclosure of Interest: None Declared

Keywords: Modified-Live vaccine, onset of immunity, PRRSV Live Vaccine

Viral and Viral Diseases
PRRS

PO-PW1-147

Beneficial impact of a PRRSV vaccination program combining a modified live vaccine and PROGRESSIS® on virus circulation and technical performance

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Introduction: PRRS is one of the most significant pig diseases in the modern swine industry. The addition of a killed vaccine (KV) (PROGRESSIS, Merial, France) vaccination at D90 of gestation in farm where sows are regularly vaccinated with a modified live vaccine (MLV) have been shown to contribute to the stabilization the sows, leading to a better control of PRRS virus circulation in the pig flows. This case report describes a long-period monitoring of PRRSV circulation using oral fluid (OF) sampling and of the technical performance, to follow up potential changes associated with an additional PROGRESSIS vaccination of sows at D90 of gestation.
Materials and Methods: On a French 400-sow PRRSV-positive farrow-to-finish farm operating in 5 farrowing batches of 80 sows each, where the routine vaccination program consisted of the use of a MLV in sows 6 days after each farrowing. Piglets were not vaccinated against PRRS. However, respiratory symptoms used to repeatedly occur and antibacterial treatments targeting PRDC were frequently applied both during the nursery and the fattening period. PRRSV infection was regularly confirmed in the nursery from 8 weeks onwards, by positive PCR results and seroconversion by ELISA (IDEXX) on sera or oral fluids (OF).
No other significant management factors were changed during the data collection. From June 2014, a booster injection at 90 days of gestation with PROGRESSIS was introduced as a routine practice. Cross-sectional collections for OF samples were performed in the pig flow 4, 7 and 16 months later and analyzed for PRRS antibodies by ELISA (IDEXX) and virus RNA presence by PCR. Technical farm data was extracted from the routine records and compared before and after the change in the vaccination protocol over two 6-month periods separated by a 6-month transition period.
Results: Following the implementation of the PROGRESSIS vaccination at 90 days of gestation, a clear increase of maternal antibodies level and a steady decreased up to early fattening were observed. Virus circulation appeared to be delayed to the fattening period, from 14weeks of life. The average number of pigs born alive increased from 13.79 to 14.57 after the implementation of the KV vaccination. Piglet mortality in the farrowing unit was reduced from 14.2% to 12.0% on average. This resulted in an increased number of weaned piglets per litter from 11.84 to 12.82. Standardized ADWG from 8 to 30 kg increased from 477 to 491 grams/day and the post weaning mortality slightly decreased from 2.6 to 2.2 %.
Conclusion: In this farm, the additional PROGRESSIS vaccination appeared to have a clear and long lasting positive effect on sow and pig performance confirmed by the changes in PRRSV infection dynamics.

Disclosure of Interest: None Declared

Keywords: Stabilization, Vaccination

Viral and Viral Diseases
PRRS

PO-PW1-090

Twenty three week duration of immunity following intradermal or intramuscular administration of PrimePac PRRS™ in 2 week old piglets

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Introduction: PrimePac PRRS™ is a newly introduced modified life PRRS Type II vaccine that can be administered either intradermally (ID) with the IDAL device or intramuscularly (IM). The objective of this study was to evaluate duration of immunity of this vaccine via both administration routes in SPF piglets vaccinated at 2 weeks of age and challenged with a virulent PRRSv strain 23 weeks later.
Materials and Methods: Two-week old piglets were vaccinated with a single dose of PrimePac PRRS vaccine (produced by MSD-AH, The Netherlands) dissolved in Diluvac Forte. Vaccinations were done in the neck with either 1 ml IM (G1; n=11) or 0.2 ml ID (G2; n=10). Piglets in a third group (G3, n=10) were non-vaccinated challenge controls. Piglets were challenged intranasally 23 weeks after vaccination with PRRSV Type II (strain VR-2332). Ten days after challenge half of the animals of each group were sacrificed for post-mortem investigation of lung lesions and remaining pigs were euthanized 28 days after challenge. Other variables measured included PRRSv serology and viremia, clinical signs, rectal temperature and weight gain.
Results: Between 3 weeks after vaccination and the time of challenge, 100% and 80% of G1 and G2 piglets were seropositive (ELISA). At challenge, serum-neutralizing antibodies (range: 1 - 5 $\log_2$) were also measured in 100% and 80% of G1 and G2 piglets and a rapid 2-fold increase occurred post-challenge. G3 pigs remained seronegative until time of challenge.
None of the pigs developed any post-challenge clinical signs. Although post-challenge rectal temperatures were not different between treatment groups, a larger increase was measured in G3 pigs. Weight gain between Day 10 and 28 was numerically higher in vaccinates than controls (G1 +0.305 kg; G2 +0.336 kg).
Post-challenge PRRSv viremia was significantly lower in G1 and G2 compared to G3 during the first 10 days post-challenge. The percent of virus positive serum samples were G1-12%, G2-20% and G3-75%.
Lung lesion scores were low ranging from 0.14±0.31 to 2.86±3.35 depending on groups. Day 10 lung lesion scores were significantly lower in G1 and G2 than G3, with odds for lower lung lesions being approx. 9 and 43 times higher in G1 and G2 than G3.
Conclusion: Intramuscular or intradermal vaccination of 2-wk old piglets with PrimePac PRRS reduced the negative effect of challenge infection with VR-2332 23 weeks after vaccination. Post-challenge viremia and gross lung lesions 10 days after challenge were reduced in vaccinated pigs irrespective of vaccination route. Although not statistically significant, weight gain was also improved in vaccinated pigs compared to the controls at all measured time points.

Disclosure of Interest: None Declared

Keywords: DURATION OF IMMUNITY, PRRS MLV vaccine

Viral and Viral Diseases
PRRS

PO-PW1-110

Efficacy of PrimePac PRRS™ following intramuscular vaccination of 5 week old pigs and challenge 4 weeks later with 2 recent PRRSv Type II isolates

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Introduction: Two studies were performed to evaluate the efficacy of PrimePac PRRS, a modified live PRRSv Type II vaccine, in piglets vaccinated intramuscularly (IM) at 5 weeks of age and challenged 4 weeks later with either SH37 or G101, 2 more recent PRRSv Type II strains.
Materials and Methods: In each study, 8 pigs (G1) were vaccinated once IM with 1 mL PrimePac PRRS (produced by MSD AH, The Netherlands) and 8 piglets (G2) served as non-vaccinated challenge control group. Four weeks after vaccination, piglets in both groups were challenged intranasally with either SH37 or G101. Piglets were observed for a 28 day period post-challenge and following parameters were measured: 1) clinical observations, 2) rectal temperatures for first 7 days only, 3) serology via IDEXX PRRS X3 Ab test, 4) viremia, post-vaccination and post-challenge nasal shedding was tested on porcine alveolar macrophages, 5) body weight and 6) histopathology of lymph nodes and lung.
Results: In both studies, G2 pigs remained seronegative until challenge, while G1 pigs seroconverted post-vaccination. Vaccine virus excretion was not detectable via nasal route on 3, 6, and 10 days after vaccination. Level and duration of post-challenge PRRSv excretion was significantly lower in G1 than G2 pigs. Post-challenge clinical signs were not observed and rectal temperatures were only slightly increased (0.1 to 0.5 °C) in T2 pigs.
Following SH37 challenge specifically, G1 pigs gained more weight at 13 dpc (+2.3 kg), and 20 dpc (+2.7 kg) than control pigs. Between Days 3 and 10 post-challenge, 1 G1 and 4 G2 pigs shed low amount of virus in nasal secretions and this shedding was no longer detectable by Day 14. The amount of virus was lower and the duration of viremia was shorter in the vaccinated animals (p=0.0013). Four out of eight G1 pigs displayed a mild pneumonia compared to 6 out of 8 G2 piglets that had a broncho-interstitial or moderate interstitial pneumonia.
Following G101 challenge specifically, G1 pigs gained more weight at 13 dpc (2.9 kg) and 20 dpc (3.5 kg) than control pigs. Between Days 3 and 7 post-challenge, 6 G1 and 4 G2 pigs shed low amounts of virus in nasal secretions but by Day 10 all animals were virus negative. The amount of virus was lower and the duration of viremia was shorter in the vaccinated animals (p=0.0031). Five out of eight G1 pigs and 6/8 G2 pigs displayed a mild interstitial pneumonia, while 2/8 G2 pigs also had a moderate interstitial pneumonia.
Conclusion: PrimePac PRRS was found safe and efficacious in pigs vaccinated at 5 weeks of age and challenged 4 weeks later with either SH37 or G101, 2 more recent PRRSv Type II isolates.

Disclosure of Interest: None Declared

Keywords: Efficacy, PRRS MLV vaccine

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-100

Determination of the frequency of animals with broadly cross-reactive neutralizing antibodies in the sow population

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Introduction: Although the mechanisms involved in PRRSV protection have not elucidated yet, the neutralizing antibodies (NA) seem to play a main role in protection against reproductive failure in sows and viremia in sows and growing pigs. However, NA developed after infection have limited ability to cross react with other isolates and might be isolate-specific. Nonetheless, a limited number of sera, known as elite neutralizers (EN), have been reported to exhibit broadly reactive NA against different isolates. The objectives of this study were to determine the frequency of sows with broadly reactive NA in different sow herds and the factors that may have an influence in the frequency of the so-called EN.
Materials and Methods: A total of 11 Spanish farms were selected based on the type of genetic used, the acclimatization protocols of in-coming gilts, the implementation of vaccination in breeding sows and the history of PRRSV outbreaks. 60 sows per farm selected on the basis of their parity (10 sows/parity) were selected for blood sampling. Sera was obtained and used in seroneutralization (SN) assays against a panel of 12 different viruses. Geometric mean of the titer (GMT) of each sera were calculated and the proportion of EN, defined as sows which sera reacted with at least 90% of the viruses used in the SN assays and whose NA GMT was equal or higher than 4 $\log_2$, was determined. Finally, the influence of the parameters considered in the NA GMT and in the frequency of elite responders was determined.
Results: The GMT in the studied population was 2.06 $\log_2$. The proportion of EN against genotype 1 viruses was 12.35% while when genotype 2 viruses were included the proportion lowered to 4.27%. However, these EN were not equally distributed among farms. Thus, in some farms no EN were found while its percentage reached 45% in others. In particular, unstable farms and farms with recent outbreaks showed a higher percentage of EN than stable farms (27.1% vs 3.8%). On the other hand, NA GMT tended to increase with sow parity in stable farms while unstable farms exhibited a more complex and variable pattern of NA GMT distribution by parity.
Conclusion: 1. The NA GMT in the studied population was lower than the minimum titer required to prevent viremia, which could help to explain the continuous outbreaks reported in sow herds.
2. The proportion of EN under field conditions is similar to that found under experimental conditions.
3. The distribution of EN is not uniform between farms. The factors which have a higher influence in the proportion of elite neutralizers and in the NA GMT are the herd status and the history of outbreaks in the farms.
4. NA GMT tends to increase with sow parity.

Disclosure of Interest: None Declared

Keywords: Elite neutralizers, Herd factors, PRRSV neutralizing serum antibody

Viral and Viral Diseases
PRRS

PO-PW1-201

Effect of Tilmicosin in the lactation diet of sows after an unplanned exposure to PRRS

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Introduction: The objective of this experiment was to evaluate the effect of tilmicosin in the lactation diet of sows on sow and litter performance after an unplanned exposure to PPRS.
Materials and Methods: One hundred ninety Sogeporc hybrid (Yorkshire-Landrace, 50:50) sows were blocked based on parity (1st, 2nd, 3rd or more), weight and P2 back fat thickness at farrowing assigned to one of two dietary treatments in a randomized complete block design. Two lactation turns were used and data were analyzed with turn as a dependent variable. Sows were fed a standard lactation diet (Control) or the Control + 200 ppm of tilmicosin. Tilmicosin was added as Tilmovet Premix (200g/kg) at 0.10% of the diet and replaced corn.
Results: Sows fed tilmicosin had greater total born 12.81 vs 13.99 ($P < 0.04$) and numerically more live born 11.52 vs 12.33 ($P < 0.17$). Sows fed diets with tilmicosin had more pigs post cross-fostering, 10.71 vs 11.96 ($P < 0.001$), suggesting that less pigs died between birth and cross fostering. Sows fed tilmicosin had more pigs post cross-fostering 11.52 vs 12.33 ($P < 0.001$). Sows fed tilmicosin had more pigs weaned as well, 10.02 vs 11.17 ($P< 0.0001$). Although this is partially because they had more born alive, it is also because they had less pigs die 1.50 vs 1.16, with the biggest difference in death loss coming prior to cross-fostering. Sows fed the Control diet weaned heavier pigs 6.92 vs 6.66 kg/hd ($P < 0.02$), but had a lighter total litter weight, 69.52 vs 74.36 kg ($P < .03$), compared to sows fed diets with tilmicosin. Weaning weight ($P < 0.02$), pig weight gain ($P < 0.02$), and pig daily gain ($P < 0.03$) all have a diet x lactation turn interaction with the first turn having a greater advantage for feeding tilmicosin over the second turn. Dietary tilmicosin improved the lactation efficiency and gain:feed of sows ($P< 0.05$). Sows fed tilmicosin lost less weight during lactation, 10.6 vs 5.2 kg. ($P < 0.05$). There was an interaction between lactation turns for sow weight loss. Sows in the first turn lost less weight during lactation when tilmicosin was added to the diet, but there was no difference in weight change during lactation between treatments in second turn, (diet x turn, $P < 0.01$).
Conclusion: Tilmicosin improved the health, livability and litter weights of the weaned pigs following an unplanned herd exposure to PRRS.

Disclosure of Interest: D. Nolan Conflict with: Huvepharma, Inc., C. Sparks Conflict with: Huvepharma, Inc., I. Cormier: None Declared, K. Herkelman: None Declared

Keywords: PRRS control, tilmicosin

Viral and Viral Diseases
PRRS

PO-PW1-098

Antigenic characterization of porcine reproductive and respiratory syndrome virus isolates by serum neutralization assays

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Introduction: PRRS genomic variability has led to the classification of the isolates into two distinct genotypes. Consistently, high antigenic heterogeneity has been reported using both polyclonal sera and monoclonal antibodies. However, there is not much information in relation to the cross-reactivity of neutralizing antibodies (NA) raised against different PRRSV isolates. The aims of this study were to characterize the NA response after infection with different PRRSV isolates, to determine the antigenic relationship between them and to establish whether antigenic and genomic classifications are consistent.
Materials and Methods: Twenty-five PRRSV isolates, 20 belonging to genotype 1 and 5 to genotype 2, were used to immunize 100 six-month-old pigs (i.e. 4 pigs/isolate). The four monospecific sera of each isolate were pooled and used in cross seroneutralization (SN) assays. Results were used to calculate antigenic similarity coefficients, to construct an antigenic dendogram and to estimate breadth (i.e. number of isolates recognized by each monospecific sera) and potency (i.e. mean titer at which PRRSV isolates were neutralized by each sera) of each sera. Finally, sequences of ORFs 2, 3, 4, 5 and 6 were determined and used to construct phylogenetic trees.
Results: Cross neutralization between isolates was generally poor. Nonetheless, breadth and potency differed significantly between sera, indicating differences in the cross reactivity of NA produced by different PRRSV isolates. Regarding the antigenic relationship between isolates, the dendrogram constructed indicates that isolates can be divided in two different serogroups, one of them comprising genotype 1 isolates and the other one genotype 2 isolates. However, no additional correlation could be determined between antigenic and genomic classification of PRRSV isolates. Nonetheless, no further serogroups could be defined within genotypes.
Conclusion: 1. PRRSV isolates differ in their ability to induce cross-reactive NA. Some isolates are capable to induce cross-reactive NA, as indicated by the high breadth and potency of the monospecific sera, while some others almost exclusively induce strain-specific NA.
2. Two serogroups, one comprising genotype 1 isolates and another one comprising genotype 2 isolates, can be defined in PRRSV on the basis of cross-reactivity in SN assays. However, antigenic relationships are not sufficient to define further serogroups within genotypes.
3.Correlation between antigenic and genomic classification can be found when genotypes are considered but antigenic relationships within genotypes do no correlate with genomic relationships based on sequences of ORFs 2, 3, 4, 5 and 6.

Disclosure of Interest: None Declared

Keywords: Antigenic groups, Neutralizing antibodies, Phylogenetic tree

Viral and Viral Diseases
PRRS

PO-PW1-178

Validation of the ID Screen PRRS Indirect ELISA

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) is a highly contagious and economically devastating porcine viral disease. The causative agent belongs to the genus *Arterivirus*, family Arteriviridea.
The ID Screen® PRRS Indirect ELISA is designed to detect antibodies directed against PRRSV in porcine serum and plasma.
This presents validation data for this test.
Materials and Methods: The ID Screen PRRS Indirect ELISA may be used with individual porcine serum or plasma (domestic swine or wild boar samples). The kit includes microplates coated with PPRSV recombinant proteins and an anti-porcine IgG horseradish peroxidase (HRP) conjugate.
Results: 1) Diagnostic specificity was evaluated on 950 samples from fattening pigs from Brittany, France. These herds had tested negative for PRRS over a number of years. 894 sera from breeders from France and other European countries were also tested. **Specificity was measured to be 99.9 % (CI95%: 99.8 % - 100.0), n=1844**.
2) 113 samples, randomly selected from infected herds in Europe, were tested in parallel with the ID Screen® ELISA and Kit A. 110 samples gave identical results on both tests. **The measured percentage of correlation was 97.3%.**
3) Eight 3-week old pigs were vaccinated intradermally with the PRRS Porcillis vaccine and challenged 31 days post vaccination with a PRRSV Brittany European strain. The 8 pigs were bled 7, 14 and 21 days post-vaccination (dpv). This study was performed by the French Reference Laboratory (ANSES, Ploufragan, France). **The ID Screen® ELISA detected seroconversion between 14 and 21 dpv.**
4) 535 sera from both disease-free and infected herds were tested in parallel using the ID Screen® ELISA and another widely used ELISA (Kit A). 530 samples gave identical results on both tests. **Measured test agreement was found to be 99.1%.**
5) The 2015 GD Deventer Proficiency Testing panel was tested. **The ID Screen® ELISA correctly identified both European and American strains**, and gave similar results to those obtained by other commercial PRRS ELISAs.
Conclusion: The ID Screen® PRRS Indirect ELISA kit demonstrates high specificity and excellent performance on reference panels. It efficiently detects positive animals in the field and correctly identified all strains.
The kit shows excellent agreement with other commercial ELISAs. With high test reproducibility and repeatability, the ID Screen® PRRS Indirect ELISA is a reliable tool for the detection of antibodies against PRRSV.

Disclosure of Interest: None Declared

Keywords: Diagnostic, ELISA, PRRS control

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-104

Dynamics of porcine reproductive and respiratory syndrome virus assessed by an oral fluid commercial PRRSV antibody enzyme-linked immunosorbent assay

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) is a major threat to the swine industry due to its financial cost. Pen-based oral fluid (OF) sampling provides an efficient monitoring method of the dynamics of PRRSV in swine populations, thus allowing the development of effective control, and/or elimination strategies.
Materials and Methods: A 10,000 PRRSV naïve-vaccinated sow farm located in Mexico, was infected with a wild type strain of PRRS in November of 2013. Infection with PRRSV was confirmed by clinical signs, PRRSV real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR), and sequencing, yielding a 1-26-2 RFLP cut pattern. A herd stabilization plan was established, i.e. sow herd mass vaccination (2X) with a modified live vaccine (MLV), nursery depopulation, and vaccination of all gilts present in the gilt development units (GDU). When herd stabilization was confirmed, as measure by the absence of clinical signs and weaning of PRRSV PCR negative gilts, a surveillance program was established using oral fluids. The IDEXX PRRS OF Ab test (IDEXX Laboratories, Inc., Westport, ME.), and qRT-PCR PRRS were used, since gilts were vaccinated at weaning. As PRRSV negative gilts populated the nursery, the same two pens per group were sampled every week from 4 weeks of age to one week before farrowing. A longer farm closure period was achieved by inseminating and gestating gilts in GDU2.
Results: During the first 40 weeks, samples tested by qRT-PCR were positive to vaccine virus (2-5-2 RFLP pattern) in gilts from 4 to 10, and 20 to 22 (re-vaccination) weeks of age. Thereafter, samples became and remained negative to PRRSV. ELISA results expressed as sample/positive (S/P) ratios were positive in gilts from 4 to 40 weeks of age, ranging from 0.6 to 3.5, with the youngest animals showing the highest S/P ratios and decreasing after 10 weeks of age. On week 40, two weeks before the new gilts farrowed, S/P ratios in OF samples increased, ranging from 2.5 to 7.3 in gilts of 22-28 weeks of age. The presence of the original PRRSV wild type virus, 1-26-2 RFLP pattern with 99% homology, was confirmed by qRT-PCR of the same OF samples.
Conclusion: Oral fluids antibody monitoring offers a stress-free and cost effective PRRSV surveillance tool, allowing immediate detection of PRRS excretion in a subpopulation(s) within a farm. Therefore, control actions can promptly be established to decrease production and economic losses. Possible reasons for this event include a non-detected PRRSV persistently infected subpopulation in conjunction with depleted immunity against the PRRSV wild type strain, and/or a biosecurity break and/or aerosol lateral contamination.

Disclosure of Interest: None Declared

Keywords: PRRS, diagnostic, oral fluid, serum, ELISA

Viral and Viral Diseases
PRRS

PO-PW1-128

Comparing algorithms performance for monitoring endemic disease: a simulation study based on the Danish PRRSV monitoring program

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Introduction: Surveillance systems are critical for accurate and timely monitoring and effective disease control. The use of statistical quality control methods for monitoring endemic diseases which are part of compulsory surveillance programs has not been previously explored. It is important to monitor changes of for instance disease prevalence, which might indicate disease spread. Thus allowing control efforts to be triggered immediately.
Materials and Methods: In this study, we investigated the performance of three univariate process monitoring control algorithms with the aim of building a monitoring system that can detect changes in the proportion of positive herds for endemic diseases in an accurate and timely way. Additionally, the effect of the sample size (or magnitude of the surveillance system) in the algorithm performance was also assessed. The Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) monitoring program in Danish breeding herds was used as model to design this study.
Three algorithms commonly used for biosurveillance were compared: Shewart P chart (PSHEW), cumulative sum (CUSUM) and exponentially weighted moving average (EWMA). In order to simulate a baseline scenario, the weekly number of positive herds was obtained from a binomial distribution with a probability (p) of 0.1 and a sample size equal to the actual number of Danish breeding herds tested for PRRSV each week from 2007 to 2014. Increases of the number of positive herds were simulated for changes in the prevalence from p=0.1 to p= 0.15 and p= 0.20 during 4, 8, 24, 52 and 104 weeks. Thereafter, the performance of the algorithms was compared by examining their detection capability under the different scenarios.
Results: The results showed that EWMA and PSHEW had higher cumulative sensitivity (CumSe) when compared with the CUSUM. Changes from 0.10 to 0.20 in sero-prevalence were easier detected (higher CumSe) when compared with changes from 0.10 to 0.15 for all three algorithms. EWMA and PSHEW detected changed showed similar results based on the median time to detection. CUSUM detected changes in the sero-prevalence later compared to EWMA and PSHEW for the different scenarios. Increasing the sample size 10 times resulted in half time to guarantee detection (CumSe=1), whereas 100 times sampled sized reduced the time to CumSe=1 by a factor of 6.
Conclusion: In summary, we showed that small changes in diseases sero-prevalence can be detected by using these algorithms. Increasing the sample size provides a faster detection for PRRS. However, the associated costs of increasing the number of herds tested and the disease should to be taken into account when making a decision.

Disclosure of Interest: None Declared

Keywords: Disease monitoring, Endemic, Univariate process monitoring control algorithms

Viral and Viral Diseases
PRRS

PO-PW1-153

Importance of PADRAP in a porcine reproductive and respiratory syndrome and porcine epidemic diarrhea regional control program in Colombia

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) and porcine epidemic diarrhea (PED) are two costly diseases affecting the swine industry. Veterinarians have developed strategies to control and eliminate these disease, however the risk of re-infection remains high as proven by the recent epidemic of PED in America. A PRRS and PED regional control program was established in the central northwestern part of Colombia. As part of this project, production animal disease risk assessment program (PADRAP) was used to identify risk factors, current management and biosecurity practices.
Materials and Methods: Seventeen farms, from the same pork association, i.e. they used the same health production, and feed services responded to the PADRAP survey. PADRAP "is an epidemiologically-based initiative to help manage disease in the swine industry. It offers risk assessment surveys and reports for measuring and benchmarking disease risks".
Results: Internal and external risk scores were, 16.92 (range: 12.52-22.8), and 26.63 (range 18.89-35.35), respectively. Scores were compared to the national Colombian internal and external risk mean, 19.02 (range 11.56-34.27) and 35.38 (range 18.57-56.54), respectively. Individual farm reports were analyzed, and a global priority action report to reduce disease transmission was completed. The main internal risks were: farrow to finish production, and short gilt acclimation period. Main external risks were: swine density, proximity to other farms, various genetic sources, unknown semen disease status, little or no sampling of replacement and biosecurity audits, as well as transport, i.e. no standardized vehicle sanitation and driver's biosecurity protocol.
Conclusion: The results of this group exercise was presented in a meeting with the majority of the responders present. Positive conclusions showed that having the same service network and standardized management, health and feeding practices reduced internal and external risk as compared to the Colombian mean. The resulting analysis also offered key external risk improvements associated to a detailed map encompassing farm location, disease status, as well as related epidemiological factors. The fact that this analysis was presented conjunctly to the group, allowed a better understanding of the swine production in this area, awareness of unknown risks, communication, education, decision making and collective commitment. Strategies such as adequate replacement acclimation, boar sampling, transport and biosecurity training sessions will be implemented; the area will re-evaluated six to eight months after implementation of these proposals.

Disclosure of Interest: None Declared

Keywords: PRRS/PED, PADRAP, Regional Control

Viral and Viral Diseases
PRRS

PO-PW1-189

ROLE OF SOW AND PIGLET VACCINATION IN STABILIZING A CLOSED HERD WITHOUT DEPOPULATION AFTER A PRRS OUTBREAK

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Introduction: Different strategies have been described to stabilize a farm after a PRRSv outbreak, with vaccination being a very effective one. This experience describes how correct use of MLV PRRS vaccination (Porcilis® PRRS, MSD AH) in sows and piglets, combined with biosecurity and management, achieves stabilization of a closed herd after a PRRSv outbreak without depopulation.
Materials and Methods: The events occurred in a PRRS(-) closed 500 sow herd. In Apr 2014, reproductive disorders such as late term abortions and early farrowings were detected. PRRSv outbreak was diagnosed by several (+) PCRs in blood samples of aborted sows. Sequencing of field strain confirmed a 95% homology with Lelystad virus. Depopulation was not a feasible option and main objective was to stabilize the farm in the shortest time possible. In May 2014, a sow mass vaccination with Porcilis® PRRS (with IDAL) was initiated; revaccination was done one month later and then maintained every 3 months. Gilt new entries were stopped for 4 months and biosecurity measures were established, as well as strict unidirectional piglet flow. Two months after revaccination, newborn piglets were checked by PCR to ensure that there were no viremic piglets (15 piglets/week). In Sep 2014, the first negative PCRs were detected in newborn piglets. After 3 consecutive weeks of negative PCRs, Strategic Piglet Vaccination was initiated (Oct 2014). Fourteen day old piglets were vaccinated with Porcilis PRRS using IDAL over a 16 week period. While Strategic Piglet Vaccination was established, PCR of 2 week old piglets were done every 2 months to ensure no new recirculation. After this 16 wk period, piglet vaccination was stopped. First non-vaccinated piglets were sampled at 3, 6 and 9 wks of age for PRRS serology and PCR.
Results: First PCR negative newborn piglets were obtained in Sep 2014, 5 months after the outbreak. Sampling was repeated every two months, and all PCRs were negative (last sampling Nov 2015). Serological results of first non-vaccinated piglets showed that there was no recirculation of PRRSv before 9 wks of age (last sampling Sep 2015). Nevertheless PRRS recirculation was detected in 12 week old piglets in the fattening units. Although this requires further investigation, this was most likely the result of a horizontal infection from pigs already present in the units.
Conclusion: The right combination of vaccination, biosecurity and management allows PRRSv stabilization after an outbreak in a short period of time without depopulation. Therefore, sows mass vaccination and Strategic Piglet Vaccination are a very effective combination to obtain PRRS negative piglets to enter the fattening units.

Disclosure of Interest: None Declared

Keywords: Biosecurity, Stabilization, Strategic Piglet Vaccination

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-152

Effects of deoxynivalenol (DON) on PRRSV immunization and challenge infection: An experimental study

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Introduction: Deoxynivalenol (DON) is a mycotoxin produced by Fusarium spp. and is a common contaminant of grains worldwide. DON has been shown to increase susceptibility to viral infections. The objective of this study was to investigate the impact of DON on porcine reproductive and respiratory syndrome virus (PRRSV) immunization and subsequent challenge infection in vivo.
Materials and Methods: In total 38 piglets were divided into four groups. Group I (10 animals) was immunized using a modified live Genotype 2 based vaccine and subsequently challenged intranasally with 2ml of a PRRSV Genotype 1 field strain containing 1 x 10^5 $TCID_{50}$/ml. Group 2 (10 animals) was similarly immunized and challenged in the presence of a diet containing 1 mg/kg DON. Group 3 (10 animals) was also similarly immunized and challenged in the presence of a diet containing 2 mg/kg DON. Group 4 (8 animals) served as infection control and was infected with the Genotype 1 field strain without prior immunization and without DON containing diet. Animals were monitored daily using a clinical score which included apathy, dyspnea, sneezing, body temperature, cyanosis, conjunctivitis, nasal and ocular discharge. Specific antibody responses were measured weekly. Blood and organ samples were used for quantification of genotype 1 and 2 viruses.
Results: Two weeks post immunization PRRSV specific antibody responses were observed in all animals of group 1 and 2. The development of PRRSV-specific antibodies was delayed in individual animals of group 3. Animals of group 4 developed the highest clinical score demonstrating the virulence of the used challenge virus. Animals of group 1 developed the lowest clinical score. In contrast, animals of group 2 and especially group 3 showed increased clinical scores almost reaching the scores of group 4. Results of the virus quantification revealed that virus loads also differed among the treatment groups.
Conclusion: The current study exemplifies that DON has marked effects on the clinical outcome of PRRSV challenge infection in prior immunized piglets.

Disclosure of Interest: None Declared

Keywords: DON, immunization (vaccination), PRRSV

Viral and Viral Diseases
PRRS

PO-PW1-103

Performance improvement after implementation of 3FLEX® with partial depopulation in a Korean farm.

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Introduction: PRRSv is a primary pathogen causing PRDC. But PRRSv can be controlled by piglet vaccination. To get the best result, the interval between vaccination and infection should be 4 to 5weeks.
The purpose of this study is to obtain a PRRS noninfection period for 4 weeks after vaccination by partial depopulation of the nursery and to evaluate the efficacy of an additional PRRS vaccination of piglets to control PRRS for the whole production period.
Materials and Methods: This farm is one-site system with 150 sows. Pigs are weaned at 25 days of age(DOA) and transferred to the grower house at 53 DOA and finisher house at 120 DOA.
Pigs in group ‘A’ are vaccinated with FLEXcombo® (CircoFLEX® and MycoFLEX®) at 3 weeks of age(WOA). In group ‘B’ and ‘C’, pigs are vaccinated with 3FLEX® (FLEXcombo® and Ingelvac® PRRS MLV) at 3 WOA.
Before implementing a partial depopulation, pigs in the nursery house were transferred to the grower house (group ‘A’). During the depopulation, newly weaned piglets were raised in the farrowing house for 4weeks (group’B’) then transferred to the grower house. After finishing partial depopulation of the nursery, newly weaned piglets were transferred to the nursery (group ‘C’).
Tissue samples from necropsy and blood samples were tested by PCR and ELISA. Mortality and clinical signs were evaluated after weaning.
Results: Nursery house mortality in ‘A’ group is 25.49%, ‘B’ group is 1.31% and ‘C’ group is 1.29%. Grower house mortality in ‘A’ group is 12.72%, ‘B’ group is 5.31% and ‘C’ group is 4.35%. Finisher house mortality in ‘A’ group is 6.03%, ‘B’ group is 2.34% and ‘C’ group is 1.82%. In group 'A', atrophy and coughing were observed. But in group 'C', there was no clinical sign in nursery. PCR results of lung tissue in group ‘A’ revealed NA type of PRRSv. PRRSv PCR positive results of blood samples in group ‘A’ were only found in blood samples of pigs 40 DOA.
Conclusion: In this study, we could demonstrate that after implementing partial depopulation, nursery pigs showed better performance and group ‘B’ pigs that had been transferred directly to the grower house, showed better performance against PRRS infection compare to group ‘A’. Partial depopulation of the nursery could control PRRSv in the nursery resulting in an improvement within a short period of time . However, there were still many pigs infected with PRRSv in the grower house. So the mortality was still high but could be controlled by using 3FLEX®. Interval between vaccination and infection was 4 to 5weeks due to partial depopulation. Partial depopulation is an important tool that can be used together with vaccination to control PRRS especially in case of early infection of PRRS after weaning

Disclosure of Interest: None Declared

Keywords: partial depopulation, piglet vaccination, PRRS

Viral and Viral Diseases
PRRS

PO-PW1-174

Efficacy test of novel PRRSV live vaccine candidate in pigs

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Introduction: The purpose of this study was to evaluate immunogenicity of WGV1014 (KCTC 12784BP) by analyzing PRRSV specific Ab titer and VN titer after challenge.
Materials and Methods: Animal. A total of 12 pigs, 3 weeks of age from a pig farm were used in the study. All pigs were confirmed to be free of PRRSV infections by use of IDEXX PRRSV X3 and VeTek™ PRRSV Detection kit. All groups of pigs were inoculated by IM route. Group 1 (n=3), 2 (n=3) and 3 (n=3) were inoculated with $10^{4.5}$ $TCID_{50}$, $10^{5.5}$ $TCID_{50}$ and $10^{6.5}$ $TCID_{50}$ of strain WG1014, respectively. Group 4 (n=3) was injected with PBS as controls. All of 4 groups were inoculated 1 time. At day 35 after vaccination, all the animals were challenged intranasally with $10^{4.5}$ $TCID_{50}$ field isolated PRRSV. Blood was taken on 0, 7, 14, 21, 28, 35, 42 and 49 days after vaccination and oral fluid samples were taken on 0, 1, 2, 3, 7, 14, 21, 28, 35, 42 and 49 days after vaccination. Serum and oral fluid samples were collected and stored at -70℃ before testing with ELISA and VN assay.
Enzyme-linked immunosorbent assay. The ELISA was performed using a IDEXX PRRS X3 as directed by the manufacturer. Serum samples were diluted 1:40 in a sample diluent. OD of each well was measured at 655 nm using a microplate reader. The presence or absence of antibody to PRRS was determined by calculating the sample to S/P ratio. Samples were considered to be positive for PRRS virus an antibody if S/P ratio > 0.4.
PRRSV VN titer assay. VN titers were determined by SN test on MARC-145 cells. A 2-fold diluted serum sample was prepared, and an equal volume of virus solution with a titer of 200 TCID50/mL was added to each dilution and incubated for 1 h at 37°C. The CPEs on the cells were analyzed for 7 days after inoculation. The VN antibody titer was defined as the reciprocal of the highest dilution that inhibited CPE in 50% of the inoculated wells.
Results: SN titer of each vaccine group was depending on concentration. After challenge, S/P ratio and neutralizing antibody titers in vaccinated animals were significantly higher to protect PRRSV infection.
Conclusion: The novel PRRSV vaccine candidate raised enough S/P ration and SN titer to prevent PRRSV infection.

Disclosure of Interest: None Declared

Keywords: Challenge, PRRS vaccine candidate, VN titer

Viral and Viral Diseases
PRRS

PO-PW1-083

A study evaluating the efficacy of Fostera PRRS in the reduction of lung lesion score following challenge with both PRRS virus genotypes 1 and 2

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Introduction: Two genotypes of porcine reproductive and respiratory syndrome virus (PRRSV) have been recognized including genotypes 1 and 2. Co-infection with both genotypes has been increasingly evident in several countries including Thailand, Vietnam, China and Korea. Although modified live vaccines (MLV) of both genotypes are commercially available, several swine herds prefer to use only one genotype of MLV. Therefore, the objectives of the study were to evaluate the efficacy of a MLV PRRSV (Fostera PRRS, Zoetis Animal Health, USA) on the reduction of clinical respiratory score, viral load and lung lesion scores following challenge with both genotypes of PRRSV.
Materials and Methods: Thirty PRRSV-free pigs were randomly allocated into 3 groups of 10 pigs each. The three treatment groups included negative control group (Neg), vaccinated group (Vac) and unvaccinated group (NoVac). Pigs in Vac group were intramuscularly vaccinated with Fostera PRRS with dosage according to the manufacturer's recommendation. Groups Neg and NoVac were left no-vaccination. At 35 days post vaccination (DPV), pigs in groups Vac and NoVac were intranasally challenged with 5 ml of the mixture of tissue culture fluid containing 10^5 $TCID_{50}$ PRRSV isolates. PRRSV isolates represent field genotype 1 and highly pathogenic PRRSV isolated from swine herds in Thailand. Pigs were evaluated for clinical respiratory score and rectal temperature were measured on daily basis. Blood samples were collected at 0, 3, 5 and 7 days post challenge (DPC). Sera were separated and assayed for the presence of virus RNA using realtime PCR. At 7 DPC, all pigs were necropsied and lung score was evaluated.
Results: Following challenged with both PRRSV genotypes, there was no pig death. Pigs in Neg group displayed no sign of clinical disease. Pigs in NoVac group had significantly higher rectal temperature compared to the Neg and Vac group. In Vac group exhibited significantly lower lung lesion score and respiratory score ($P<0.05$) compared to NoVac group. In addition, the Vac group had significantly lower level of viral load of both genotypes 1 and 2 in serum following challenge.
Conclusion: The results of the study demonstrated the efficacy of Fostera PRRS in the reduction of clinical respiratory disease, viral load and lung lesion score following challenge with PRRSV genotypes 1 and 2, compared to non-vaccinated groups.The results of the study demonstrated the efficacy of Fostera PRRS in the reduction of clinical respiratory disease, viral load and lung lesion score following challenge with PRRSV genotypes 1 and 2, compared to non-vaccinated groups.

Disclosure of Interest: None Declared

Keywords: Co-infection, Modified-Live vaccine, Porcine reproductive and respiratory syndrome virus (PRRSV)

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-148

Vaccination with Ingelvac PRRS MLV for PRDC control in Korean swine farm

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Introduction: PRDC is regarded as one of the most serious problems in worldwide swine production recently. There are many pathogens involved in PRDC such as PRRSV, PCV2, SIV, MH, HPS, APP, and PM etc. PRRSV among them could cause disease on its own, and cause immunosuppression of infected pigs, resulting in increased susceptibility to secondary bacterial infection. The main purpose of this study was to reduce mortality and to increase performance using Ingelvac PRRS MLV in Korean swine farm that was affected by PRDC.
Materials and Methods: This field study was conducted in a 250 sow two-site farm located in Korea. In this farm situation, breeders and nursery pigs are on one site and finishers on another site. After weaning, pigs stay in site 1 until 90 days old and transferred to site 2. Transferred pigs are naïve pigs against PRRSV. Even though PRDC occurred in site 2, pigs in site 1 were continuously confirmed as PRRSVnegative.
Affected pigs in site 2 showed diverse clinical signs including lethargy, anorexia, coughing, labored
breathing and death. Because their poor growth performance led to overcrowding in the barn, more
complex problems occurred. For that reason, antibiotics and feed additives were supplied to site 2, but PRDC was not solved. After traditional treatments with antibiotics and feed additives, mortality rate slightly decreased temporarily. After 5 month Ingelvac PRRS MLV vaccination was initiated. To obtain best performance of vaccine, all nursery pigs in site 1 were vaccinated at 3-4 weeks before moving to site 2. Mortality rate in site 2 was checked monthly.
Results: Before traditional treatments, mortality rate was above 13%. Mortality rate reduced to 5% temporarily after traditional treatments. However, mortality rate increased up to more than 10% after a few months After Ingelvac PRRS MLV vaccination of pigs in site 1, average mortality rate in site 2 went down to 2%. Not only did the clinical signs largely disappear, the pigs in site 2 also showed improved growth performance, which helped to solve an overcrowding problem.
Conclusion: In this case where PRDC was caused by PRRSV as the primary pathogen, controlling of PRRSV infection by implementation of vaccination strategy, was the main solution to improve clinical disease. The vaccination strategy also improved production performance. In general different methods can be applied to control respiratory diseases. PRRS MLV vaccination is an important part of the solution when PRRS is involved in a PRDC problem. A strategy of PRRSV MLV vaccination at 3-4 weeks before PRRSV infection is a promising method for providing sufficient immunity to pigs against PRRSV.

Disclosure of Interest: None Declared

Keywords: piglet vaccination, PRDC, PRRS

Viral and Viral Diseases
PRRS

PO-PW1-175

Serologic evidence of porcine reproductive and respiratory syndrome virus infection in farm and abattoir pigs in Nigeria

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) is considered the most important viral disease of intensive swine production because of the severe economic losses associated with it. Generally, the disease causes reproductive failure characterized by infertility, mummifications, abortions, still-births and birth of weak piglets in sows, and respiratory distress in piglets and growing pigs. There are several studies on the occurrence of PRRS in pig herds in North America, Central and South America, Europe and Asia. However, apart from few reports from South Africa, there is lack of information on the disease in Africa. In particular, the status of PRRS in Nigeria remains largely unknown despite reports of mummifications, abortions, still-births and respiratory disorders in pig herds in the country. Hence, we conducted a serological survey in pig farms and abattoirs in Lagos and Oyo states, southwest Nigeria to determine the prevalence of the disease.
Materials and Methods: 368 pig sera (162 males, 206 females) collected between May, 2014 and July, 2015 were screened using a commercial enzyme-linked immunosorbent assay (ELISA) kit designed to detect antibodies to both genotypes (types 1 and 2) of PRRS virus (PRRSV). The sampled pigs were observed for clinical symptoms and the farmers interviewed on herd health history.
Results: An overall PRRSV antibody prevalence of 53.8% (198/368) was obtained. Group seroprevalence was 58.6% (95/162) and 50.0% (103/206) for farm and abattoir pigs, respectively while there was a significant difference in seropositivity between pigs from Lagos (89.4%) and Oyo (48.6%) states. Common clinical symptoms observed, especially on the farms, include loss of appetite, fever, lethargy, emaciation, nasal and ocular discharges, enlarged lymph nodes, wobbly gait, paddling movement, variation in litter size, respiratory distress and history of piglet deaths shortly after birth.
Conclusion: Since pigs are currently not vaccinated against PRRS in Nigeria, the findings of this study suggest that the sampled pigs were infected with PRRSV. Considering the persistent nature of the virus and its reputation as a cause of severe economic losses in the swine industry, our findings necessitate continuous surveillance for the disease among pig herds in Nigeria. This will help to ascertain the actual burden and increase awareness of the disease in order to facilitate its early detection. Among other control measures, the introduction of pigs of unknown serological status into breeding herds should be discouraged. Also, further studies to isolate and identify PRRSV strains circulating in Nigeria are advocated.

Disclosure of Interest: None Declared

Keywords: Farm and abattoir pigs, Nigeria, Porcine reproductive and respiratory syndrome

Viral and Viral Diseases
PRRS

PO-PW1-134

Efficacy of 3FLEX® for the control of PRRS infection in the nursery at a Jeju island swine farm

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Introduction: PRRS infection is one of the most serious diseases that causes huge economic loss. The purpose of this study is to evaluate 3FLEX® vaccination to control PRRSv infection in the nursery and to compare the efficacy when vaccinated separately or as 3FLEX®, in the combination with FLEXcombo®.
Materials and Methods: The study was performed in a farrow to finish one site farm of 200 sows. The farm used already FLEXcombo® (CircoFLEX®+MycoFLEX®) in piglets at 21days of age(DOA). Weaned piglets at 40~70 DOA suffered from PRRSv, so Ingelvac® PRRS MLV was added to the vaccination schedule.
PRRS MLV vaccination schedules were 3FLEX (CircoFLEX + MycoFLEX + Ingelvac PRRS MLV) or
FLEXcombo (CircoFLEX + MycoFLEX) + Ingelvac PRRS MLV separately per group and evaluated
separately. Groups A (20 pigs) and B (19 pigs) served as controls with FLEXcombo vaccination only. Group C (23 pigs) received FLEXcombo + Ingelvac PRRS MLV separately and finally group D (23 pigs) received 3FLEX. Evaluated parameters were weights, average daily weight gain (ADG) and mortality of each group over a period of 2 weeks starting with weaning at 32 DOA.
Results: Before the start of the study, this farm suffered from decreased productivity. Pigs in groups A and B showed symptoms of a PRRS infection with apathy, reduced appetite, fever and reduced weight gain. 3 pigs in group A and 1 pig in group B group died and both groups needed immediate medications (Ceftiofur, Prednisolone and Sulpyrine). The investigation of blood samples demonstrated PRRS at the age of 40 days. After medication, there was no additional mortality in pigs of groups A and B over the 2weeks experimental period. No pigs died in groups C and D. In addition, there were no clinical symptoms of PRRS and pigs grew well. Additional PRRS vaccination (group C and D) substantially increased performance with regard to mortality, ADG and FCR and there was an additional benefit in ADG(average of C and D group was about 50g higher) and FCR(average of C and D group was about 0.22 lower) when using 3FLEX instead of separate injection of FLEXcombo and the PRRS vaccine.
Conclusion: Generally suow vaccination may prevent vertical transmission of PRRS from sow to piglets but cannot prevent horizontal transmission in nursery or growfinishing. To control clinical disease in pigs after weaning, a piglet vaccination has to be applied. Additional PRRS vaccination in piglets has demonstrated high efficacy in controlling PRRS in piglets with 3FLEX being the most effective and
economic schedule. Beside demonstrating best production data it saves labor and reduces stress for the piglets by reduced number of injections compared to separate injections of FLEXcombo and Ingelvac PRRS MLV.

Disclosure of Interest: None Declared

Keywords: 3FLEX®, FLEXcombo®, PRRS

Viral and Viral Diseases
PRRS

PO-PW1-123

Comparison of viremia of type I and II porcine reproductive and respiratory syndrome virus in Taiwan

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Introduction: Porcine respiratory and reproductive syndrome virus (PRRSV) is an important porcine pathogen that causing huge economic impact in swine industry worldwide. The genetic characteristics of the PRRSV strains clearly indicate the existence of two major genotypes, the European type (EU genotype, type 1) and the North American type (NA genotype, type 2). However there was no viremia detection result of type 1 and 2 PRRSV by quantitative real-time PCR assays in Taiwan. The aim of this study was to compare viremia of type 1 and 2 PRRSV from Taiwanese pigs.
Materials and Methods: Serum samples were collected from 273 sows, 270 suckling pigs (≦3-week-old), 837 nursery pigs (4- to 12-week-old) and 144 growing pigs (≧13-week-old) from middle and southern Taiwan from August 2014 to December 2015. All serum samples were RNA extracted followed by reverse transcription and quantified the type 1 and 2 PRRSV load by zip nucleic acid probes based real-time PCR. The correlation between type 1 and 2 PRRSV viremia percentages and ratio of groups in viremic pigs were evaluated using the chi-square test with Yate's correction. *P* values <0.001 were considered highly significant.
Results: The PRRSV viremia percentage of all collected samples was 33.99% (518/1524). The type 1 and 2 PRRSV and co-infection viremia percentage were 2.03% (31/1524), 31.69% (483/1524) and 0.26% (4/1524), respectively. In viremic pigs, type 1 and 2 PRRSV detection rate were 6.76% (35/518) and 94.02% (487/518), respectively. The ratio of sow, suckling, nursery and growing pig groups in viremia pigs were 3.67% (19/518), 11.97% (62/518), 81.47% (422/518) and 2.9% (15/518), respectively. The detection rate of type 1 PRRSV of viremic sow, suckling, nursery and growing pig groups were 2.86% (1/35), 22.86% (8/35), 74.29% (26/35) and 0% (0/35), respectively. The detection rate of type 2 PRRSV of viremic sow, suckling, nursery and growing pig groups were 3.7% (18/487), 11.09% (54/487), 82.14% (400/487) and 3.08% (15/487), respectively. Type 2 PRRSV viremia percentage of all samples and different groups was highly significantly than type 1 PRRSV (*P* <0.0001). The ratio of nursery pig group in viremic pigs was highly significantly than other groups (*P* <0.0001).
Conclusion: The results of this study indicate that despite type 2 PRRSV was dominant (93.24%), type 1 PRRSV still existed in Taiwan and nursery pigs were prevailing PRRSV infective group.

Disclosure of Interest: None Declared

Keywords: European type porcine respiratory and reproductive syndrome virus, North American type porcine respiratory and reproductive syndrome virus, Viremia

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-139

PRRSv prevalence follow up of a structured PRRSv control program from a large specialized swine veterinary practice

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Introduction: In 2014, the need for a structured PRRS control program in The Netherlands was explained because of clinical problems, economic losses and possible changes in PRRS dynamics A unified approach to diagnosis, monitoring, biosecurity measures and targeted vaccination are major requirements for successful control. As Lintjeshof swine vets cover a substantial number of Dutch pig farms, many of the conditions are easily unified and monitored. A unified PRRS diagnostic and monitoring system similar to MSD-AH ResPig® system was rolled out on the Lintjeshof farms in 2014. Multiplying herds were categorized for PRRSv based on AASV system. PRRSv prevalence for weaners and nursery pigs between 2014 and 2015 was studied and compared with figures indicative for the average of the Dutch swine industry.
Materials and Methods: The ResPig program includes PRRSv serological and PCR investigations every 4-6 month. On 25 Lintjeshof farms, PRRS antibody titers were measured in sows, weaners and 10 week-old piglets. PRRSv PCR was tested on saliva from recently weaned and 10 week-old piglets. Since Lintjeshof uses the same protocols, the PRRSv prevalance. of weaned and nursery batches was calculated and compared with the Dutch average, as well as the proportion of multiplying farms in the various PRRSv categories: Cat I(PRRSv unstable) are sero(+) sows and PCR(+) weaners, Cat II(PRRSv stable) are sero(+) sows and PCR(-) weaners, Cat III are PRRSv sero(-) sows and PCR(-) weaners.
Results: For 2014 and 2015, Lintjeshof PRRSv prevalence at weaning was 20% vs 13% (Dutch average: 14% vs 15%) and 40% vs 30% (Dutch average: 36% vs 35%) at end of nursery. Multiplication farms were categorized as no farms in CatIII and increase in CatII farms from 80% in 2014 to 90% in 2015. In that time, CatI decreased from 20 to 10 %.
Conclusion: The structured approach resulted in a PRRSv prevalence at weaning below the Dutch average, a trend also reflected in the amount of PRRSv stable sow herds (CatII). The actual PRRSv field virus prevalence is probably lower because vaccinated piglets are also sampled. Still 20% of PRRSv negative weaned batches get infected in the nurseries, indicating the importance of good biosecurity procedures. No farms were in CatIII due to the high vaccination rates of sows which can contribute to PRRS stability in multiplying farms.
A uniform PRRSv approach is essential to control and monitor PRRSv on farms. PRRS related problems need to be addressed and strong and weak points regarding PRRS control were discussed upon evaluation of results. Such discussions are needed to convince farmers about implementing improved biosecurity and targeted vaccination to control PRRS.

Disclosure of Interest: None Declared

Keywords: PRRS control, PRRS prevalence, ResPig

Viral and Viral Diseases
PRRS

PO-PW1-160

Evolution of PRRS prevalence and evaluation of circulating PRRS strains based on ORF-5 in the Netherlands.

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Introduction: MSD-AH`s ResPig® offers the opportunity to investigate the PRRS status via cross-sectional blood sampling of gilts, sows, weaners and nursery pigs, and saliva testing (via PCR) of two animal groups every six months. PRRS PCR results of weaners can give an indication of the PRRS stability of the sow herd at the time of sampling. PCR results of the oldest nursery pigs provide information about the status of pigs entering the finishing units/farms. In addition, positive PCR test results can be sequenced if requested. The aim of this study was to investigate the 2015 PRRS prevalence in Dutch farms and their ORF-5 sequence and compare the results with those of the previous year.
Materials and Methods: In 2015, 80 ResPig® investigations with saliva samples coming from weaners and 10 week old piglets were tested. Saliva samples from each group were analyzed via qPCR and then classified as negative or positive. Per veterinarians' request, 13 positive PCR samples were ORF-5 sequenced (IVD Hannover). PRRSv farm prevalence of weaners and oldest nursery pigs was calculated and compared with the results of 2014
Results: The prevalence of PRRS at weaning was 15% (12/80) versus 14% in 2014. The prevalence of PRRS at the end of the nursery period totaled 35% (28/80) versus 36% in 2014. More specific investigations indicated that 32% (22/68) of investigations had PRRSv-negative weaned piglets but PRRSv positive nursery pigs
Thirteen (13) positive PCR results were sequenced. The ORF-5 sequence of positive saliva samples varied from 86% to > 98% homology with the Lelystad strain: 86% (1), 87% (3), 88% (1), 92% (1), 93% (1), 94% (1), 96% (1), DV (4). The DV-strain (vaccine Porcilis® PRRS) was only isolated from Porcilis PRRS vaccinating farms
Conclusion: The 2015 PRRS prevalence at weaning and at the end of the nursery is still rather low and remained at the same level as 2014 . The actual PRRSv field virus prevalence is probably lower because vaccinated piglets were also sampled. Thirty (30) % of the sequenced samples were only DV positive and came from Porcilis® PRRS vaccinating farms

About 32% of the farms with PRRS negative weaned piglets had PRRS positive pigs at the end of the nursery. This stresses the importance of a correct internal biosecurity within PRRS control on farms (in addition to vaccination). Compared to previous years, there are no indications that there was a further genetic drift to more heterologous PRRS strains in 2015. This hypothesis needs further investigation in the future.

Disclosure of Interest: None Declared

Keywords: prevalence, PRRS, qPCR

Viral and Viral Diseases
PRRS

PO-PW1-113

Use of a modelling approach to coordinate PRRS control decisions

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Introduction: The porcine respiratory and reproductive syndrome (PRRS) is a major viral disease in swine production. In most cases, farmers decide whether to control this disease or not within their herd on a voluntary basis. Nevertheless, individual decisions have an impact on the risk for other farmers to be infected. Since some farmers are grouped in associations/geographical areas, it is relevant to investigate how a group of farmers can coordinate individual decisions, implementing incentives for disease management. The objective of this study is to develop a framework to propose an optimal strategy to be used by the decision maker of an association, strategy which consists on rules to define at each time step which incentives should be used.
Materials and Methods: We describe an approach to propose control strategies which are adaptive to the evolution of the epidemiological situation over time. We assumed that the collective decision-maker should at each time-step select the incentive to optimise a criterion, for example the minimisation of the total cost at the group level (incentive, control and disease costs). The decision-maker can choose among various incentives levels, ranging from cheap no-incentive to costly incentives. We assumed that the decision-maker knows how the farmers would react to its incentives. A Markov decision model was defined including stochastic compartmental models representing PRRS virus spread within a group of farms among which some are PRRS virus positive. For each incentive level, the proportions of farmers implementing controls measures was introduced into the model avoiding the formalisation of individual decisions. The model was solved to produce a strategy optimising the total cost at the group level. For comparison, we simulated the spread of the PRRS virus within the group of herds in 2 situations : (1) when the collective decision-maker uses the optimal strategy and (2) when no incentive was used by the collective decision maker over time .
Results: We obtained a strategy corresponding to a guideline indicating at each time step the action to use according to the observed epidemiological situation. This guideline is translated into a decision-tree. When using the optimal strategy, various levels of incentive are used over time inducing an average total cost at the group level lower than if we systematically used each incentive level.
Conclusion: We propose a complex strategy optimising the total cost which is translated in simple rules ("if-then"). While optimising the total cost, the model can be extended to consider also an objective in terms of prevalence decrease.

Disclosure of Interest: None Declared

Keywords: decision, modelling, PRRS

Viral and Viral Diseases
PRRS

PO-PW1-126

MHC down-regulation on PRRSV-infected porcine macrophages does not completely inhibit virus-specific cytotoxic T-cell responses

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Introduction: PRRSV infects macrophages in pigs using CD163 and CD169 as receptors. The replication of this virus has an immunomodulatory effect in pigs, resulting in weak and delayed adaptive immune responses. Persistence of PRRSV in lymphoid tissues after acute viremia and clinical disease indicates that the virus may have a mechanism to subvert or suppress immune defenses. Down-regulation of MHC class I and II molecules has been reported as a characteristic change on PRRSV-infected macrophages. However, the kinetics and impact of this process on presentation of PRRSV epitopes to T-cells and cytotoxicity have not been clearly studied. To define the impact of MHC class I and II down-regulation, cytotoxic T-cell responses against PRRSV-infected macrophages were evaluated.
Materials and Methods: Porcine alveolar macrophages (PAMs) were collected from the lungs of healthy PRRSV-free 14 day old piglets. To assess the down-regulation of MHC class I, II and SWC on PAMs by PRRSV, the synchronized infection of PRRSV into PAMs was carried out using a magnetic nanoparticle (ViroMag™, OZ biosciences, France) and concentrated $PRRSV_{SD23983}$. Cytotoxic T-cell response against PRRSV-infected monocyte-derived macrophages (MDMs) stained with TFL4 (OncoImmune, USA) was evaluated using PantoxiLux (OncoImmune), a cell permeable, fluorogenic substrate that detects both granzyme B and upstream caspase activities. Surface staining of cells and flow cytometry analysis were performed to measure expression level and/or phenotype of CD4, CD8, CD172a, TFL4, PS, and MHC class I and II molecules on PAMs and MDMs. Intracellular staining for PRRSV N protein was also performed to determine the infectivity of PAMs and MDMs.
Results: Using autologous MDMs infected with $PRRSV_{SD23983}$ for 20 hours, we determined the cytotoxic responses of T-cells (33 days post-challenge) collected from an intranasally PRRSV-infected and clinically recovered gilt. Positive intracellular PRRSV N staining (ICS) was evident in 12.3 to 15.6% of MDMs at 20 hpm, with clear down-regulation of MHC class I (-57.7%) and II (-62.5%) (Figure 1). PRRSV-infected MDMs with moderate down-regulation of MHC class I and II molecules were still capable of presenting epitopes to cytotoxic T-cells. The major phenotype of cytotoxic T-cells was $CD8^+$ with a minority of cells with $CD4^+$ phenotype.
Conclusion: These results suggest that PRRSV replication in porcine macrophages does not completely suppress T cell-mediated immunity, including CTL. Therefore, PRRSV epitopes presented in both early and late cycles of virus replication may contribute to potentially protective immunity.

Disclosure of Interest: None Declared

Keywords: PRRSV, MHC down-regulation, cytotoxic T-lymphocyte response

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-173

PRRS Immune Response in Vaccinated and non-Vaccinated Naïve Pigs

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Introduction: Immuno-modulation of pig immune response to Porcine Reproductive and Respiratory Syndrome virus (PRRSv) infection has been reported, particularly the inhibition of interferon-g (IFN-g) and increasing the number of interleukin10 (IL-10) producing cells. Thus, one of the strategies in controlling the immune-modulation of PRRSv is to vaccinate piglets for PRRS immune response. The purpose of this study was to investigate the immune response using serum neutralizing (SN) antibody titer and specific IFN-g producing peripheral blood mononuclear cells (PBMC) to PRRSv in vaccinated and non-vaccinated naïve pigs in Thailand.
Materials and Methods: Eighteen, 21-day-old, male piglets, bought from a PRRS-free herd, were individually housed and divided into 3 groups, (n=6 per group); A (non-vaccinated, no challenged), B (non-vaccinated, challenged) and C (vaccinated with challenged) groups. The C group was vaccinated with the PRRS vaccine (Ingelvac PRRS® MLV, BI, Germany) at 28 days of age (D0). The B and C groups were challenged with 2 ml of field PRRSv (10^5TCID$_{50}$/ml) and 10^7TCID$_{50}$/ml intramuscularly on day 28 and 42 post vaccination, respectively. Blood samples were collected on day 0, 28, 35, 42 and 49 for serum neutralizing antibodies and PRRS specific IFN-g producing PBMC, measured by flow cytometry (BD Accuri™ C6).
Results: The SN titer response showed that non-vaccinated pigs had no response, meanwhile the vaccinated pigs had small response on D35 and stronger response after 2nd challenged. Moreover, the percentage of specific IFN-g producing PBMC had responded right after vaccination and drastically increase at the 1st challenged then recovered on D42. After the 2nd challenged, both B and C groups showed the same trend in response. No response in the negative group (A group).
Conclusion: The percentage of specific IFN-g producing PBMC, after PRRSv challenging, had increased drastically in vaccinated pigs compared to the slower response of SN titer. However, after the 2nd challenged, the response in specific IFN-g producing PBMC showed the same trend either vaccinated or non-vaccinated pigs, but not the same response in SN titer. In conclusion, the protective immune response to the PRRS infection should be intensively focused on IFN-g producing cells than the SN titer, particularly in vaccinated piglets.

Disclosure of Interest: None Declared

Keywords: immune response , naïve pigs, PRRS control

Viral and Viral Diseases
PRRS

PO-PW1-093

Evaluation of a new commercially available PRRSV vaccine upon exposure to a recently isolated Belgian genotype 1, subtype 1 PRRSV strain (Flanders 13)

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Introduction: Recently a new live attenuated porcine reproductive and respiratory syndrome virus (PRRSV) vaccine became commercially available on the European market (Ingelvac PRRSFLEX EU, Boehringer Ingelheim). This vaccine is based on a genotype 1 subtype 1 strain. Subtype 1 strains cause reproductive disorders in breeders and limited respiratory problems in young pigs. In 2013, a more pathogenic genotype 1, subtype 1 strain was isolated from weaned pigs showing respiratory disorders on a Belgian farm (Flanders 13). In the present study, the degree of clinical and virological protection was assessed in pigs vaccinated with Ingelvac PRRSFLEX EU and challenged six weeks later with Flanders 13.
Materials and Methods: Twelve 4-weeks-old piglets from a PRRSV-negative farm were divided in 2 groups. Group Vac was vaccinated intramuscularly with Ingelvac PRRSFLEX EU and group Unvac was left unvaccinated. All piglets were challenged intranasally with PRRSV strain Flanders 13 (2 ml, 10^5 TCID$_{50}$/dose) six weeks after (mock) vaccination. Blood was collected weekly during the experiment to follow the immune response (IPMA). After challenge, the animals were observed clinically for body temperature and respiratory disorders. Blood and nasal secretions were collected at challenge and at different days post challenge (dpc) in order to monitor viral replication and nasal shedding.
Results: All vaccinated pigs seroconverted within two weeks after vaccination, whereas the control animals remained seronegative until challenge. Fever and other signs of disease were minimally observed in both groups upon challenge. After the peak viral load, a significant reduction in PRRSV titer was observed in the vaccinated group during the second week post challenge. The area under the curve (AUC) was significantly reduced in vaccinated animals compared to unvaccinated pigs, while the duration was slightly reduced. All pigs shed virus at 3 dpc with significantly lower PRRSV titers in nasal secretions in vaccinated group compared to unvaccinated controls. At 7 dpc, the mean PRRSV titer in vaccinated animals was also significantly lower. Vaccination shortened the duration of nasal shedding post challenge with 6.16 days and the mean virus titers in the nasal secretions (AUC) were significantly lower in vaccinated group compared to the unvaccinated group.
Conclusion: A sufficient protection was observed upon challenge with subtype 1 PRRSV strain Flanders 13 in pigs vaccinated with the new live attenuated vaccine Ingelvac PRRSFLEX EU. Viremia and nasal shedding of PRRSV Flanders 13 were significantly reduced (duration and viral titers) in vaccinated animals.

Disclosure of Interest: C. Bonckaert Conflict with: Boehringer Ingelheim paid for the study, C. Kraft: None Declared, G. Cluydts: None Declared, H. Nauwynck Conflict with: Boehringer Ingelheim paid for the study

Keywords: None

Viral and Viral Diseases
PRRS

PO-PW1-130

Monitoring of European PRRSV strains using sequencing technologies

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) is considered one of the most economically important infectious diseases of swine. PRRSV is divided into European (Type I) and American (Type II) genotypes. PRRS is caused by a single stranded positive-sense RNA enveloped virus with a high mutation rate leading to greater heterogeneity of the nucleotide sequence between the individual strains. The high genetic virus diversity increases the risk of reduced sensitivity for real-time RT PCR diagnostic tool. The aim of the present study was to monitor circulating PRRSV strain throughout Europe using capillary ORF7 sequencing and NGS technology.
Materials and Methods: Thermo Fisher Scientific established several collaborations with laboratories and research institutes to collect field samples. More than 120 PRRSV positive samples were collected in different countries (Spain, Belgium, Netherlands, Czech Republic, Slovenia and Russia) and were sequenced in our lab. According to PRRS viral load, two strategies were performed. Samples with high PRRS viral load were sequenced using NGS workflow on Ion Torrent™ PGM™ instrument, to obtain whole genome sequences. Bioinformatics analysis is performed using an in-house developed tool, Viral Genome Assembly Pipeline (VGAP) for genome assembly and identification of a consensus sequence. For samples with low PRRS viral load, ORF7 sequencing was performed using capillary electrophoresis. Therefore, 16 samples were sequenced using NGS workflow and 33 samples were sequenced in ORF7 sequencing.
Results: 33 ORF7 sequences obtained were aligned with PRRS-EU1 (28/33) and PRRS-EU2 (5/33) reference sequences and were confirmed by BLAST analysis. Whole genome sequencing data on 16 samples, were of high quality with a mean coverage of ~4000X. A mean nucleotide read length is around 110pb. Mapping reads against PRRSV genomes available in public database highlighted different European reference strains: subtype 1 (KF203132, GQ461593, KT159248) and subtype 2 strains (KP889243). Comparison between sequences obtained showed an important variability of PRRSV strains on ORF7 fragment as well as on PRRSV complete genome. Mutations on primers/probes design can lead to important impact on detection and decrease kit's performances: low fluorescence level, late or absence of detection.
Conclusion: The monitoring of circulating strains, associated with participation to Proficiency Testing Scheme, are necessary to identify and sequence new variants. New NGS technology enables to sequence the complete genome of PRRSV and is really essential to develop accurate diagnostic tools. Thermo Fisher Scientific offers different workflows for NGS and targeted sequencing to enable this monitoring.

Disclosure of Interest: None Declared

Keywords: PRRSV, Sequencing

Viral and Viral Diseases
PRRS

PO-PW1-171

Coinfection of PRRSV and Streptococcus suis in an Intensive Farm in China

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) and *Streptococcus suis* are common pathogens in pigs causing large economic loss to world pig industry. Field studies showed that PRRSV was usually detected with other pathogens. A case report was performed in this study to give more insights into coinfections of PRRSV and *Streptococcus suis*.
Materials and Methods: In December, 2015, an intensive pig farm with 330 sows in Hubei province was reported to be suffered from acute death of nursery pigs with a mortality of 22% and lasted for a few month. Infected pigs showed neurological symptoms. Four sicked nursery pigs were necropsied, and tissues (including brain, lung and tonsil) were collected to detect pathogens. RNA was extracted from lung and tonsil to detect PRRSV and HCV, while DNA was extracted from brain and lymph to test for PRV and PCV2. PRRSV ORF5 gene was subsequently sequenced to analyze the genetic relationship with virulent virus strain. In addition, the serum samples were tested for the gE antibodies of PRV by commercial ELISA (IDEXX). Moreover, bacterial was isolated and test for susceptibility of antibiotics.
Results: The nursery pigs at the age of 40-50 day old showed neurological symptoms, such as twitch and stroke. Some pigs died acutely. Morbidity reached 30%, while the mortality was about 22%. Anatomy results indicated that the most dominant pathological lesions were pericarditis, peritonitis and meningeal hyperemia. All the samples were positive for variant PRRSV. Nsp-2 deletion was confirmed by RT-PCR. ORF5-based sequence alignment and phylogenetic analysis showed that this strain had 84.3%>92.7% identity with current commercial attenuated vaccine strains in China, while showed the highest similarity to JXA1-R (99% identity). On the other hand, one sample showed gD of PRV positive in PCR, while ELISA analysis of gE antibody were negative, indicating the vaccination of herd with gE-gene deleted pseudorabies vaccine, not wild type pseudorabies virus infection.
Streptococcus suis were isolated from both 4 lungs. Antimicrobial susceptibility test results showed that the isolated S. suis was resistant to cefradine, Streptomycin and azithromycin, while susceptive to ceftriaxone, amoxicillin and ampicillin.
Conclusion: In this study, the coinfection of PRRSV and S. suis was diagnosed. Also, no history of vaccination against PRRS was used in this farm, the virus might be transmitted into the farm through weak biosafety. To stabilize the disease, the farm was suggested to vaccinate herd with JAX1-R vaccine and, simultaneously mediated with sensitive drugs.
Acknowledgement
This work was supported by China Agricultural Research System (CARS-36).

Disclosure of Interest: None Declared

Keywords: coinfection, PRRSV, S. suis

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-131

Porcine reproductive and respiratory syndrome virus challenge alters jejunum chemosensing mRNA abundance in grower pigs

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Introduction: Chemosensing in the gut involves G protein-coupled receptors and their associated G proteins, including taste 1 receptors (T1Rs) and taste 2 receptors (T2Rs). Sweet taste is recognized by T1R2+T1R3 heterodimers and umami flavors by T1R1+T1R3 heterodimers; however, bitter perception is detected by an array of T2Rs. Advances in gastrointestinal chemosensing have uncovered mechanisms by which specific nutrient and pathogen components evoke multiple neuroendocrine and metabolic responses that alter appetite and innate immunity. However, nothing is known about how these taste receptors are regulated during pathogenic challenges in pigs. Symptoms of porcine reproductive and respiratory syndrome virus (PRRSV) in growing pigs often include suppressed growth rates and feed intake. Therefore, the objective of this study was to determine the extent to which jejunum bitter and sweet taste receptor mRNA abundance changes during a PRRSV challenge in grower pigs.
Materials and Methods: Sixteen mixed-sex pigs approximately ten weeks-of-age and naïve for PRRSV were allotted into 2 treatments: sham (Controls) and PRRSV inoculated. Pigs in the PRRSV group were intranasally inoculated with 10^5 $TCID_{50}$/ml of a 1-7-4 PRRS virus isolate (day post inoculation (dpi) 0). At dpi 10, all pigs were euthanized. At necropsy, jejunum sections were isolated, flushed with saline and mucosal scrapings collected. Total RNA was isolated from mucosal scraping, cDNA synthesized and quantitative real-time PCR used to determine the mRNA abundance of nutrient transporters and taste receptors.
Results: Compared to the Controls, PRRSV reduced ($P < 0.001$) glucose transporter 2 (GLUT2) mRNA abundance (1.00 vs. 0.27, respectively), but not sodium dependent glucose transporter 1 (SGLT1, $P = 0.50$). Sweet taste receptor T1R1 mRNA abundance was also reduced ($P = 0.020$) in PRRSV compared to Controls (0.52 vs 1.00, respectively); however, there was only a tendency ($P < 0.10$) for PRRSV to reduce T1R2 and T1R3 mRNA abundance. There was no difference ($P > 0.10$) in bitter taste receptor mRNA for T2R38, T2R9 or T2R4 abundance on dpi 10 of PRRSV infection. The fatty acid receptor, GPR120, which has been shown to have anti-inflammatory, insulin-sensitizing and glucagon like peptide-1 promoting effects was down regulated ($P < 0.001$) due to PRRSV compared to Controls (0.46 vs 1.00, respectively). The short chain fatty acid receptor, GPR41, mRNA did not differ ($P = 0.24$) between groups.
Conclusion: In conclusion, PRRSV challenged pigs had significant reductions in jejunum T1Rs, GLUT2 and GPR120 mRNA abundance. This may indicate changes in intestinal chemosensing induced by PRRSV challenge or could be reflective of reduced feed intake.

Disclosure of Interest: None Declared

Keywords: chemosensing, intestine, Porcine Reproductive and Respiratory Syndrome virus

Viral and Viral Diseases
PRRS

PO-PW1-182

Effect of Dual Technology Prime Boost vaccination in sows on circulation of PRRSV in post weaning piglets

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Introduction: PRRS is one of the most significant pig diseases in the modern swine industry. Current vaccination strategies are based on the use of Modified Live Vaccines (MLV) and/or Inactivated Vaccines. Nevertheless, in many farms PRRSV circulation in weaned piglets remains a clinical problem. The objective of this study was to evaluate the effect of implementation of a Dual Technology Prime Boost (DTPB) vaccination combining MLV and PROGRESSIS® (Merial) at the end of gestation to increase the colostral immunity and to delay post-weaning PRRSV circulation.
Materials and Methods: On a Dutch farrow-to-finish farm of 1000 sows, piglets showed clinical problems linked to PRRSV between 4 and 10 weeks of age (woa) confirmed by PCR analysis on broncho-alveolar lavage fluid (BALF) from 4 woa onwards. The vaccination program consisted of the use of an EU type MLV in sows at 60 days of gestation (dog) and at 6 days after farrowing. The piglets were not vaccinated against PRRS. It was replaced by a vaccination with the MLV at 60 dog and a booster with PROGRESSIS at 90 dog.
During 1 year after the implementation of the new program, cross-sectional collections of BALF samples were performed in 4 to 10 woa piglets and analyzed for PRRS RNA by PCR.
Blood samples of 6 sows were taken at 4, 3 and 1 week *ante partum* for IDEXX ELISA evaluation. For each sow, 3 piglets were randomly selected for blood sampling at 4, 7 and 10 woa. Samples were tested by IDEXX ELISA and by PCR for viral RNA presence.
Clinical problems and technical farm data were monitored before and after the change in vaccination protocol.
Results: The analysis in weaned piglets showed PRRSV presence in BALF and in sera from 10 woa after implementing the DTPB program.
Sows showed an anti-PRRSV antibody titer decrease between 4 and 3 weeks *ante partum* and a strong increase after Progressis vaccination. A high correlation between antibody levels in sows 1 week *ante partum* and 4 woa piglets was observed showing good colostrum management. Antibodies in piglets steadily decreased from 4 to 10 woa, indicative for the absence of field infection with PRRSV.
The clinical problems in the weaned piglets clearly decreased. Technical performance of the herd improved for several parameters: number of weaned piglets per year increased from 28.2 to 31.0, number of live born piglets increased from 32.2 to 34.7. Post weaning growth increased from 302 to 320 grams/day and mortality decreased from 4.7 to 0.8 %.
Conclusion: In this study, implementation of the PROGRESSIS vaccination at the end of gestation in the DTPB concept delayed the PRRSV circulation within the weaned piglets up to 10 woa. Technical performance of the farm and clinical signs of PRRSV infection clearly improved.

Disclosure of Interest: J. Spaans: None Declared, A. Verhaegen Conflict with: Merial B.V., V. Dekens Conflict with: Merial B.V., H. Smits Conflict with: Merial B.V., T. Meyns Conflict with: Merial N.V., F. Joisel Conflict with: Merial S.A.S.

Keywords: efficacy, stabilization, vaccination

Viral and Viral Diseases
PRRS

PO-PW1-144

Cell-mediated immune responses against the PRRS virus in gilts vaccinated with UNISTRAIN® PRRS or ERYSENG® PARVO combined with UNISTRAIN® PRRS

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Introduction: Current knowledge of *Porcine Reproductive and Respiratory Syndrome* virus (PRRSV) immunology is still limited; however, it seems clear that modified live vaccines (MLV) are a reasonable choice for the immunization of pigs. After PRRSV MLV vaccination, cell-mediated immune (CMI) responses could be responsible for limiting the duration of viraemia, and consequently the spread of the virus. UNISTRAIN® PRRS is a commercial PRRS MLV which can be combined with ERYSENG® PARVO (vaccine against *Porcine parvovirus* (PPV) and *Erysipelothrix rhusiopathiae* (SE)). HIPRA has registered this combination, proving the viability of the PRRSV after mixing, the safety and the efficacy of this combination against PRRSV, PPV and SE. However, there is no data available about the CMI response of this combination.
The objective of the present study was to assess the CMI response against heterologous PRRSV isolates in gilts vaccinated with UNISTRAIN® PRRS and in gilts vaccinated with the combination of UNISTRAIN®PRRS and ERYSENG® PARVO.
Materials and Methods: Sixteen PRRS-naïve, healthy gilts, 6-month-old, were randomly divided into three groups: group A (UNISTRAIN® PRRS + ERYSENG® PARVO, 2 ml/dose), group B (UNISTRAIN® PRRS, 2ml/dose) and group C (control group, 2 ml PBS/dose). All the animals received the treatment of their group at D0 and D21 of the study. For the safety assessment, body temperature and local and general clinical signs were evaluated. Blood samples were collected at days 0, 21, 28 and 42 of the study. Heterologous CMI responses were measured by IFN-γ ELISPOT against three well-known strains: two European -type I- (3262 and 3267) and one North American -type II- (VR-2332). The homologous CMI response was also evaluated using the vaccine strain. PRRS virus antibodies were evaluated by ELISA to ensure that vaccinated animals seroconverted.
Results: The safety of the vaccination protocols was demonstrated in groups A and B. Before vaccination, all the samples were negative for PRRSV antibodies. After vaccination, seroconversion by ELISA was demonstrated in all the immunized pigs, whereas the control animals remained negative throughout the experiment. Regarding CMI, the results showed a higher response in groups A and B compared to group C ($P<0.05$) for all the evaluated PRRSV strains. When the vaccinated groups were compared, the CMI response was similar throughout the experiment except for day 28 (A>B for all the evaluated strains; $P<0.05$).
Conclusion: The present study suggests that UNISTRAIN® PRRS administered alone or combined with ERYSENG® PARVO induces a significant specific CMI response by stimulating early IFN-γ producing cells against heterologous and homologous PRRS virus strains.

Disclosure of Interest: None Declared

Keywords: None

Viral and Viral Diseases
PRRS

PO-PW1-185

Antiviral Activity of Tilmicosin against Highly Pathogenic Porcine Reproductive and Respiratory Syndrome (HP-PRRS) isolated during 2009 to 2015

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Introduction: Highly Pathogenic Porcine Reproductive and Respiratory Syndrome (HP-PRRS) is currently widespread in China and several Southeast Asian countries. Management, biosecurity and vaccination have been used to encountered with PRRS virus outbreaks. However, immune evasion strategies and various antigenic heterogeneities of the causative viruses could hamper those strategies. Nowadays, successful of controlling procedures is still limit. One of alternative ways to control the virus in the field is chemotherapeutic. Tilmicosin, a tylosin derivative macrolide antibiotic which is recommended for treatment and prevention of respiratory disease associated with bacterial infection in pig, can induce positive impacts in PRRS infected herds and reduce type 2 PRRS virus infection *in vitro*. However, it is known that some viral gene may experience mutagenesis or antigenic change under antibodies or drug pressure. In this study, minimum inhibitory concentration of tilmicosin to HP-PRRS virus which were isolated from several provinces in Northeastern part of Thailand during 2009 to 2015 were tested.
Materials and Methods: MARC-145 cell lines were maintained in Dulbecco's Modified Eagle Medium. The virus used in this study were HP-PRRS virus which were isolated from infected pigs in the Northeastern part of Thailand in year 2009, 2010, 2011, 2012, 2014 and 2015. Some of those farms added tilmicosin in pig feed to control or prevent PRRS virus in their farm. Tilmicosin used in this study was obtained from Huvepharma Thailand.
Twenty thousand MARC-145 cells/well were seeded in 96-well micro-titer plates and infected with PRRS virus at MOI of 0.05. Tilmicosin was then treated to infected cells 24 hours later. The minimum inhibitory concentration (MIC) of tilmicosin which protected 50 percent of cells to produce CPE were recorded and calculated using Reed and Muench's formula.
Results: The mean MIC of tilmicosin to HP-PRRS virus isolated in year 2009, 2010, 2011, 2012, 2014 and 2015 were 26.33±0.47 (n=2), 26.46±0.20 (n=8), 26.17±0.28 (n=3), 26.5 (n=1), 26.50(n=1) and 26±0 (n=2) µg/ml respectively.
Conclusion: Tilmicosin has been added to pig feed aiming to control clinical sign of PRRS/HP-PRRS virus in pig farm ever since. However, tilmicosin inhibit PRRS/HP-PRRS virus in a dose-dependent manner. Under low concentration of tilmicosin, viral replication would be inhibited partially. The genetic mutation of PRRS virus could occur in that condition and could lead to the resistance of the virus to tilmicosin. Our result indicates that no resistance of HP-PRRS virus to tilmicosin have been developed. However, further study in genetic level is still needed.

Disclosure of Interest: None Declared

Keywords: PRRS control, tilmicosin

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-091

PHYLOGENETIC ANALYSIS OF TYPE I PRRSV STRAINS FROM POLAND AND HUNGARY: NEW INSIGHTS ON ORIGIN AND DIVERSITY

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Introduction: The available information about PRRSV diversity from Central Europe is limited and has never been analyzed in a systematic way. The aim of the study was to analyze nucleotide sequences of ORF5 of PRRSV strains from Poland and Hungary and reveal their origin in these countries.
Materials and Methods: Complete ORF5 sequences from Hungary and Poland were obtained for this study as well as sequences previously submitted to GenBank have been compared to a set of ORF5 sequences of PRRSV Type 1 originating from Europe, North America and Asia. In total, 92 sequences from Hungary from 2003–2014, and 86 from Poland from 1994–2014, were analyzed in a set of 861 ORF5s of PRRSV Type 1, using maximum-likelihood method.
Results: As expected, ORF5 sequences of PRRSV Type 1 were clustered into 4 genetic subtypes. In globally distributed and most common genetic subtype 1, 9 genetic lineages were identified. All sequences originating from Hungary and Poland belonged to subtype 1. In Hungary most of the sequences were from lineages 1 and 2, including 58.7% and 32.6% of all the sequences in this coutry, respectively. Several recent sequences belonged to lineages 3, 4, 5 and 6.In Poland strains of lineage 2 and 5 were the most common, including 41.9% and 36.0% of all the sequences of the country. Fewer sequences belonged to lineages 1, 3 and 8.Sequences before 2003 were not available in Hungary, but the oldest sequences from Poland from 1994–1996 belonged to three different lineages (2, 5, 8) suggesting multiple sources of the virus introduction. From the early introductions only lineages 2 and 5 are present today while the lineage 8 disappeared. Interestingly the strains of lineage 1 (represented by Lelystad virus), present in Hungary at least from 2003, were detected in Poland only since 2011.
Conclusion: The reconstruction of the history of PRRSV in a country is only possible if numerous sequences from different time periods are available. At least three factors contribute to PRRSV diversity in a country: movement of infected animals (or semen), the use of MLVs, and the evolution of the resident and new strains. The analysis of EUROSTAT data showed unidirectional trade of live pigs from the Netherlands, Germany or Denmark to Poland and Hungary which may explain the origin of PRRSV. The impact of the MLVs on genetic diversity is difficult to assess. In Poland sequences of ORF5 nearly identical to Amervac (HIPRA) were detected in early 1990-ties while the vaccine was developed and licensed around 10 years later. On the other hand lineage 1 where Porcilis PRRS (MSD) is clustered appeared in Poland in 2011, several years after the vaccine was licensed.

Disclosure of Interest: None Declared

Keywords: PRRSV, ORF5, Diversity, Origin

Viral and Viral Diseases
PRRS

PO-PW1-096

Safety and Efficacy of a novel modified live PRRSV vaccine in bred gilts and sows

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) can be devastating to breeding herds, due to losses resulting from reproductive failure as well as delayed return to estrus. Additionally, piglets born to affected dams suffer from increased weakness at birth, poor growth performance, increased susceptibility to respiratory infections, as well as higher pre-weaning mortality. Vaccination prior to breeding is considered to be of value for decreasing these detrimental effects.
Materials and Methods: Two blinded, completely randomized studies were conducted to establish safety (Study 1) and efficacy (Study 2) of a new European-derived, PRRSV modified live virus (MLV) vaccine; ReproCyc® PRRS EU. A total of 110 non-bred PRRSV negative commercial mixed breed gilts were used in Study 1 and 2, respectively. In Study 1, vaccinates received a 10x overdose of the MLV vaccine while control gilts received a placebo on Days 0 and 14 of the study. All gilts were 90 + 3 days of gestation at the time of first vaccination and were clinically healthy. In Study 2, two vaccinate groups (2 and 3) received a low and high titer of the MLV vaccine while the control gilts (groups 1 and 4) received a placebo approx. 28 days prior to breeding on Day 0 of the study. On Day 118 (approx. 90 days of gestation), groups 1, 2 and 3 received a virulent heterologous European PRRSV challenge (Isolate 205817). Gilts in both studies were observed for local and systemic reactions following treatment. Primary safety and efficacy criteria included percentages of live, stillborn and mummified piglets per litter at farrowing and live piglets, piglet viremia and viral load, piglet clinical signs and ADWG at weaning.
Results: In Study 1, Gilts had no systemic reactions to vaccination and viremia. There were no relevant differences for live born piglets, general health during the suckling period, ADWG, piglets at weaning, lung pathology, and viral load in lung tissue. In Study 2, vaccinated gilts had positive serological responses as early as 14 days post-vaccination and lower incidence of viremia following challenge. Vaccinated gilts had more healthy live born and weaned piglets per litter with higher ADWG. Vaccinates had fewer weak live and mummified piglets per litter, less piglets with abnormal clinical signs and viremia.
Conclusion: The results from these studies are supportive of the clinical safety and efficacy of ReproCyc® PRRS EU in breeding females against virulent PRRSV infection. It was shown that administration of one IM dose of the novel MLV vaccine to gilts approximately 1 month prior to breeding is a viable option for preventing production losses associated with PRRSV.

Disclosure of Interest: None Declared

Keywords: PRRS MLV vaccine

Viral and Viral Diseases
PRRS

PO-PW1-095

ANALYSIS OF THE RECENT RE INTRODUCTION OF PRRSV IN CHILE

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSv) type 2 was first detected in Chile in1999. The country self-declared PRRSv negative in February of 2013, after 11 years of eradication program. However, in October 2013, PRRSv was detected again. The first case was reported in a sow farm located in an area of high pig density. Clinical disease was severe and production was greatly affected. This event was followed by several outbreaks, where most of the pig production is located. This study summarizes the progress of the PRRS epidemic and conducted a phylogenetic study to understand the origins and transmission of PRRSv within the country.
Materials and Methods: The data from the PRRS national eradication program, launched in 2014, was used to describe the epidemic. Also, samples from all affected sites were obtained. Representative samples were sequenced in addition to obtaining sequences provided by the Chilean Association of Pork Producers. A total of 26 sequences obtained between October 2013 and April 2015 were analyzed. We added to the analysis, the closest sequences found in NCBI public nucleotide database as well as reference strains isolated in Chile in the previous PRRS introduction. To reconstruct the phylogeny, we used a Bayesian approach, specifying a constant population size tree prior and an exponential relaxed clock. The analysis was run for $5x10^8$ iterations. The final tree was annotated and the trees sampled before convergence were burned. Final estimates of tree parameters its high posterior densities (HPD) were obtained from the annotated tree.
Results: By the end of 2014, out of the 2,368 known pig sites (backyard & commercial) present in the country, 98 had been diagnosed with PRRSv infection. Forty three of these belonged to commercial pig sites and 55 to backyard pigs.
We found that viral sequences were closely related with 2012 and 2013 North American viruses, and very distant (~85% identity) to previous Chilean PRRSv. Our analysis suggest that Chilean 2013-2015 PRRS viruses had a common ancestor in May 2012 (95%HPD Oct 2011-Jan 2013), and shared a common ancestor with the closest related sequence (PRRSV2/Indiana/XW079/2013) in April 2011 (95%HPD February 2010-May 2012).
Conclusion: The results show that viruses that affected Chile in 2013 were different from previous PRRSv indicating a new viral introduction. Additionally, time to common ancestor of Chilean viruses suggests that these viruses may have evolved in Chilean pigs at least couple months before being detected in the first commercial farm. Phylogenetic analysis has proven a useful tool to help elucidate the source of the 2013 outbreak in Chile.
This study has been partially funded by FONDEF ID14I10201

Disclosure of Interest: None Declared

Keywords: genetic diversity, PRRS, PRRS outbreak

Viral and Viral Diseases
PRRS

PO-PW1-132

An antibody response against structural proteins of porcine reproductive and respiratory syndrome (PRRS) virus after the experimental infection

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Introduction: PRRS is one of the most economically important viral diseases in swine industry. The genome of the causative virus encodes seven structural proteins, namely, GP2a, GP2b, GP3, GP4, GP5, M and N. Although the antibody response against N protein after the infection has been well investigated using an available kit, the antibody response against other structural proteins is yet to be evaluated. Here, except for GP2b, we evaluated by an immunofluorescence assay-based method the antibody response against each structural protein after an experimental infection.
Materials and Methods: The genes encoding ORF2-ORF7 in the field-isolated PRRSV (INP-002; American type belongs to Cluster III) were cloned into an expression vector and transfected into HEK293 cells. Eight piglets at 35 days of age were nasally infected with the above PRRSV. The serum sample was collected at week 0, 2, 3 and 4 post infection (p.i.). The serum samples were serially diluted from 20 to 1,280-fold and incubated with the HEK293 cells expressing each structural protein. The antibody reacting to the structural protein was labeled with FITC-conjugated secondary antibody and detected by a flow cytometer. The copy number of virus RNA in the serum was determined by real-time PCR.
Results: A strong antibody response (≥1280-fold dilution of the serum) against N protein was detected in six of eight piglets from week-2 p.i. and it was detected from week-3 p.i. in the remaining piglets. The responses against other structural proteins were detected in most piglets from week-3 p.i. except for the one against GP4. The antibody against GP4 was detected only at week-4 p.i. in seven piglets but the antibody titre in five of them was very low (≤40-fold dilution). The antibody response against GP2a was observed only in three piglets. In two piglets, the response against GP5 was not observed. Nonetheless, the viral RNA was decreased in these two piglets in the same manner as with the other piglets. Half of the piglets showed a strong response against M protein from week-3 p.i., while the remaining half at week-4 p.i.. The copy number of viral RNA at week-4 p.i. was markedly smaller in the piglets showing an earlier response against M protein compared with the others showing a later response.
Conclusion: Antibodies against GP2a and GP4 seem to be not always produced upon PRRSV infection. The antibody against GP5 is one of the major antibodies induced by infection but it is seemingly not a unique antibody possessing a neutralizing activity. It is of interest that piglets quickly producing the antibody against M protein showed a quicker decrease of viral RNA in serum. The antibody against M protein may play a role in the clearance of PRRSV.

Disclosure of Interest: None Declared

Keywords: Antibody response, immunofluorescence assay (IFA), Structural protein

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-158

Molecular characterization and phylogenetic analysis of PRRSV strains in Greek swine farms

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) is widespread among swine population, causing reproductive disorders in sows and respiratory disease in pigs of all ages. In Greece, PRRSV was first detected in 1993. Today, PRRSV appears in an enzootic form, with elevations and declines of reproductive problems in breeding stock and increases of respiratory problems in growing-finishing pigs, causing economic losses. The aim of this study is to detect and molecularly characterize PRRSV strains which circulate in Greek swine farms.
Materials and Methods: During 2012-15, over 1460 blood serum samples were obtained from pigs residing in 50 commercial pig farms of Greece with previous history of PRRS. The specimens underwent RNA isolation, and extracts were tested with two genotype-specific real-time RT-PCR protocols (North American / European). DNA sequencing was performed in RT-PCR-positive samples from 13 farms, aiming to molecularly characterize ORF 4. Moreover, post-mortem examination was performed in dead piglets derived from the aforementioned farms. Tissues from various organs were obtained for histopatholgoical testing, as well as PRRSV antigen detection via immunohistochemistry.
Results: The results of the molecular testing indicated that only the European genotype was circulating, in 70% of the tested farms. Additionally, analysis of nine ORF 4 sequences which were obtained revealed the presence of 6 distinct phylogenetic clades, with large phylogenetic distances. The variability of the neutralizing epitopes of Gp4 protein was high in the obtained sequences. Gross pathology and histopathological findings were compatible to those of PRRS. Immunochistochemical testing showed presence of viral antigens in lymphoid tissues and lungs.
Conclusion: The present study comprises the first report on PRRSV molecular characterization in Greece. Although only European genotype strains were detected in all tested farms, the high variability of the strains raises questions regarding their introduction in the farms, as well as for the protection offered by commercial vaccines.

Disclosure of Interest: S. Chaintoutis Conflict with: Zoetis Inc., V. Papatsiros Conflict with: Zoetis Inc., G. Brellou Conflict with: Zoetis Inc., E. Tzika Conflict with: Zoetis Inc., I. Tsakmakidis Conflict with: Zoetis Inc., I. Vlemmas Conflict with: Zoetis Inc., V. Psychas Conflict with: Zoetis Inc., C. Dovas Conflict with: Zoetis Inc.

Keywords: Greece, Molecular characterization , PRRSV

Viral and Viral Diseases
PRRS

PO-PW1-202

Effect of the B1 fumonisin : injuries histopatologíc lesions in pig kidney and identification of the of the PRRS virus.

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Introduction: The FAO (1999) estimates that at least 25 % of world grain and seed production is contaminated by fungi and their mycotoxins, and are considered as one of the greatest risks that affect human and animal health. The fumonisins have been associated with certain diseases in animals such as the leucoencefalomalacia in equines (ELEM) and pig lung edema (PLE). The porcine reproductive and respiratory syndrome (vPRRS) has economically impacted the national and international swine industry, for 20 years.
Materials and Methods: The reference Strain ATCC 2332 of the PRRS virus was cultivated in MA-104 African Green monkey kidney cells, and the pigs were challenged with 10^4 $TCFF_{50}$ /ml. The FB1 used was a standard (SIGMA), in 5 mg and 10 mg vials with 98 % purity. A stock solution was prepared at a concentration of 87ppm in distilled water. The FB1was administered to weaned pigs in 12ppm (mg/kg live-weight) by the oral route by means of a probe. Experimental animals: Twenty five recently weaned pigs 22-36 Days of age, with a weight of 4.17 to 7.6 kg, from a vPRRS free farm were used. The animals were distributed into 5 groups; each group consisting of five pigs **Group A**: negative Control. **Group B**: Treated with FB1 12 ppm on day 0 (beginning of the experiment). **Group C**: inoculated with PRRSv on day 8. **Group D**: inoculated with vPRRS on day 0 and simultaneously intoxicated with FB1 12ppm. day 0 day 0. **Group E:** intoxicated with FB1 12ppm on day 0 and inoculated with vPRRS on day 8. Blood was collected in vacuum tubes containing EDTA on days 0, 8, 16 and 18, for processing by the nested RT-PCR technique to identify the PRRSv. For the histopathological study samples of kidney were collected and fixed in 10 % buffered formalin for further processing (4) and finally stained with hematoxylin and eosin.
Results: The kidney lesions observed are suggestive of toxic processes, apparently in proximal convoluted tubules, that can relate to the presence of FB1, so we suggest conducting trials to evaluate these effect. Glomerulonephritis and the PRRSv can lead to a hipoproteínemia and to the development of edema. In Group C, where only PRRSv was applied, we also noted kidney injuries, it was noteworthy since the presence of these alterations in this pathology is not common. It has been reported kidney lesions associated with infection of the PRRSv, showing inflammatory infiltration observed in renal cortex and Medulla, as well as renal vascular changes.
Conclusion: We need to focus an investigation that concentrates in assessment of renal damage in pigs by FB1

Disclosure of Interest: None Declared

Keywords: fomonisin, PRRS, lession

Viral and Viral Diseases
PRRS

PO-PW1-159

Histopathological and molecular findings in emaciated pigs from Mexico

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Introduction: Wasting of post-weaning piglets is a problem of global pig farming. The most important viral diseases linked to this problem in Mexico are porcine circovirus type 2 (PCV2)-systemic disease, porcine reproductive and respiratory syndrome (PRRS) and blue eye disease (BED); the latter, is only present in Mexico. These diseases cause great economic losses and predispose to the development of co-infections, mainly with bacteria. The objective of this work was to identify microscopic lesions and involvement of PCV2, PRRS virus (PRRSV) and porcine rubulavirus (PoRV) in emaciated piglets.
Materials and Methods: Thirty-seven necropsies of pigs with poor body condition were performed in the *Bajío* region of Mexico. Inclusion criteria were 6-16 week-old pigs displaying marked growth retardation (body condition scoring 1 or 2). Tissue samples of lung and lymph nodes were fixed in buffered formalin for histopathological analysis and immunohistochemistry for PCV2. Same tissue samples were taken and frozen for subsequent detection of PCV2 by PCR and PRRSV and PoRV by RT-PCRs.
Results: Histopathologically, interstitial pneumonia was observed in 25/37 (68%) of the piglets, catarrhal-purulent bronchopneumonia was observed in 7/37 pigs, broncho-interstitial pneumonia was found in 5/37 pigs and 2/37 animals had fibrino-hemorrhagic-necrotizing pleuropneumonia. Also, a varying degree of lymphocyte depletion in lymphoid organs was found in 14/37 (38%) animals. A total of 16/37 (43%) pigs were PCV2 PCR positive, 18/37 (49%) yielded a positive RT-PCR for PRRSV (North American strain) and 1/37 (3%) pigs positive for PoRV. PCV2 and PRRSV co-infection was noticed in 7/37 (19%) piglets, while only 1/37 (3%) was positive both for PRRSV and PoRV. Finally, 10/37 (27%) animals were negative for all three viruses.
Conclusion: In this study, histopathological and viral detection methods showed that 73% of wasted pigs were associated with infections or co-infections with PRRSV and PCV2. It is important to highlight that 38% of pigs had pulmonary lesions suggestive of bacterial infections. It is possible that potential immunosuppression or immunomodulation of viral origin (PCV2 and/or PRRSV mainly) favored the development of these infections. In conclusion, results obtained in this study suggest that PCV2 and PRRSV are present very often (alone or in co-infection) in wasted pigs from Mexican farms. The PoRV played a relatively minimal role in the causation of emaciation in the studied pigs.

Disclosure of Interest: None Declared

Keywords: Mexico, piglets, wasting

Viral and Viral Diseases
PRRS

PO-PW1-179

PRRS Dual Technology vaccination program (MLV/KV): virus circulation monitoring in pig flows using oral fluid sampling

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) is endemic in major swine producing countries. Dual-technology (DT) vaccination programs combining modified live vaccine (MLV) and killed vaccine (KV) have gained interest in order to better control PRRSV infection and favor whole herd stabilization. A survey for PRRSV circulation in the pig flow on eight farms having implemented a DT vaccination program in sows was performed by monitoring the PRRSV serological and virological status from the nursery to late fattening stages.

Materials and Methods: In these farms, the initial routine PRRSV vaccination scheme included a type-1 PRRSV MLV used for primo-immunization in quarantine followed by booster injections at D6 of lactation or at D60 of gestation or blanket vaccination every 4 months. The piglets were not vaccinated against PRRSV. PROGRESSIS sow vaccination at D90 of gestation was added to the routine of the herd. PRRSV infection and antibody profiles were monitored using oral fluid (OF) sample collection in the pig flows. Samples were assayed for PRRSV antibody level using a commercial kit (Idexx PRRS OF Ab Test) and viral RNA presence was assessed using a RT-PCR technique (LSI VetMAX™). Eight transversal surveys and 14 longitudinal follow-ups of 3 to 5 OF samples per survey or follow-up were performed. Data was collected prior to and following the implementation of the KV additional injections in sows for a total of 81 OF samples.
Results: The percentages of OF positive for viral RNA in pigs aged 5-7, 8-10, 11-13, 14-16 and more than 17 weeks were 17%, 20%, 40%, 84%, 40%, respectively, before the implementation of the KV vaccination and 0%, 0%, 0%, 38% and 60%, respectively, for pigs born from KV-boosted sows. Virus circulation appeared to be delayed to mid-fattening, i.e. from week 14 of life and later in pigs born from KV-boosted sows.
In OF, the antibody levels from piglets born from MLV-only-vaccinated sows were low as early as a few weeks following weaning, thus confirming lower MDA transfer and subsequently high susceptibility to PRRSV infection. Pigs born from the KV-boosted dams showed a significantly higher anti-PRRSV ELISA antibody level just after weaning ($p<0.05$), which steadily decreased up to 11 to 16 weeks of age depending on the batch. In these pigs, the seroconversion indicative of virus circulation appeared to be consistently delayed to a few weeks following the entry of the pigs in the fattening unit.
Conclusion: These results suggest that the addition to a PRRS MLV program of a PROGRESSIS vaccination in sows around 3 weeks before farrowing can be an efficient tool to better control early virus circulation in pigs and to lead to a whole herd stabilization.

Disclosure of Interest: B. Boivent Conflict with: Merial S.A.S., O. Merdy Conflict with: Merial S.A.S., G. Perreul Conflict with: Merial S.A.S., J.-B. Hérin Conflict with: Merial S.A.S., F. Joisel Conflict with: Merial S.A.S.

Keywords: oral fluid, vaccination, whole herd stabilization

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-108

Field sample evaluation of the "adapted for oral fluids" PRRS X3 ELISA protocol compared to the commercial PRRS Oral Fluids ELISA

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Introduction: The commercial PRRS Oral Fluids ELISA is a highly sensitive assay, particularly when detecting PRRSV exposure through antibody monitoring in large swine populations. Prior to the launch of this commercial assay, a modified protocol was developed for oral fluids using the existing PRRSV antibody serum test (PRRS X3). Although the modified test also has excellent specificity and sensitivity, the protocol requires overnight incubation and titration of a non-standard conjugate. This study evaluated the performance of these two assays, using identical samples submitted to 3 different laboratories.
Materials and Methods: A field study was performed to compare test results from oral fluid field samples using the USDA licensed IDEXX PRRS OF Test (OF) and a PRRS X3 modified protocol (ON) for oral fluid samples. Two hundred and ninety four expected antibody negative oral fluid samples from 34 field locations of a single production system were tested in two laboratories (A and C), and a subset (264) were tested at a third laboratory (B). Samples were tested within 1-5 days of collection in laboratories A and C, and after 1 freeze-thaw cycle at laboratory B. Laboratories A and B performed the ON protocol, while laboratory C performed testing using the commercial IDEXX PRRS OF Test. All three laboratories used the published cut off of S/P ≥ 0.40, and all three centrifuged samples prior to testing. For Laboratory A, S/P values between 0.20 and 0.40 were considered suspect and therefore additionally defined.
Results: Negative (S/P < 0.4) results were obtained in 292, 264 and 272 of the oral fluid samples from Laboratories A, B and C, respectively. An additional 5.4% of Laboratory C S/P results ranged between 0.4 and 0.7.
After follow-up sampling and re-testing, 3 of the 34 expected negative field locations were determined to be seroconverting. These 3 locations represented 48 samples of which the OF Test detected 10 positive, whereas the ON protocol in Laboratory A detected only 2 positive and 2 suspects, at S/P ~0.2. Outside of the one confirmed seroconverting nursery, there were 9 other nursery sites represented. Age at sampling and transition diet make up could not be confirmed, therefore detection of residual maternal antibodies (several nursery sources were PRRS positive stable sow farms) and/or the impact of sprayed dried plasma cannot be ruled out.
Conclusion: The commercial PRRS OF kit is a highly sensitive test which was able to detect early seroconversion before the modified ON protocol. There was also numerical variation noted between results of the labs performing the ON protocol, which may have been influenced by the non-commercial adaptations to the PRRS X3 kit for OF.

Disclosure of Interest: None Declared

Keywords: PRRS, oral fluids, monitoring

Viral and Viral Diseases
PRRS

PO-PW1-193

The first case report of PRRSV and PCV2 infection in Albania

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV)and Porcine -2 (PCV2) infections remain major causes of significant economic losses in pig production worldwide. During the last yearsnew pig farms were established in Albania, considering the increasing demand of the meat processing industry. No published data existregarding PRRSV and PCV2 infections in Albania.
Materials and Methods: The present study reports on a breeding stock of a farrow-to-finish commercial pig farm, located in South Albania (Mursi, coordinates: 39° 42' 17" North, 20° 4' 36" East). The capacity of the farm was 120 sows under production (commercial hybrids of Large White × Landrace), introducing gilts France, and Greece. The farm's vaccination scheme for breeding stock and weanersdid not includeimmunizationsagainst PRRSV and PCV2.
The farm suffered from increased mortality rate, poor growth performance, severe respiratory signs weaning and growing/finishing stage. The acute respiratory disease in weaners included coughing, sneezing, increased respiratory rates, dyspnea ("thumping"),nasal and eye discharges, lethargic and hairy wasting pigs. The clinical signs of sows were characterized form sporadic premature farrowingswith increased number of stillbirth and weak piglets, moderate inappetence and anorexia.
On November 2015 blood samples were obtained from 8 sows (4 lactating and 4 dry period sows), 5 piglets of 15-20 days of age, 20 piglets of 40-70 days of age (4 of 50 days, 4 of 60 days, 4 of 70 days) and 5 of 130-150 days of age. In addition tissues samples were collected from weaners (e.g. lymph nodes lung, kidney, liver) for histopathological exams.
Blood serum samples underwent nucleic acid extraction. Extracts were examined by: real-time RT-PCR (qRT-PCR) for PRRSV (type 1-PRRSV EU and type 2-PRRSV US), and by real-time PCR for PCV2.
Results: Testing of sows was negative for both viruses.Pigs from 15 days to 130-140 days of age were positive for PRRSV. In addition, PCV2 viraemia was detected in pigs from 50 days to 130-140 days of age.
The histopathological findings were compatible with PRRSV and PCV2 infection.
In conclusion, the clinical signs and losses of this clinical casewere due to PRRSV and PCV2 co-infection.
Conclusion: This is the first report of PRRSV and PCV2 infection in Albania. The recent introduction of unvaccinated weaned pigs and gilts from other European countries without adequate biosecurity measures (e.g. quarantine), and the absence of vaccination program against PRRSV and PCV2 are the possible causes of this outbreak of PRRSV and PCV2 co-infection.

Disclosure of Interest: None Declared

Keywords: PRRS, PCV2, pig

Viral and Viral Diseases
PRRS

PO-PW1-170

GENETIC DIVERSITY OF PRRSV COLOMBIAN STRAINS ISOLATED BETWEEN 1998 AND 2014

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) is a causative agent of reproductive failure in sows and respiratory disorders in pigs. It was recognized in Colombia since 1996. PRRSV shows a high degree of genetic variation and some antigenic heterogeneity. ORF5, the gene encoding the major envelope glycoprotein is the standard sequencing target and it is a valuable tool to understand what is happening in the field. The purpose of this study was to conduct a phylogenetic analysis of ORF5 sequences obtained from different pig Colombian production regions between 1998 and 2014.
Materials and Methods: Samples were collected from different infected swine farms located in seven Colombian departments where pig production is well developed. The obtained ORF5 sequences were compared using 534 nucleotides from this gene. A dendogram was constructed using 55 strains and 71 reference sequences published in the gene bank which represented families from both genotypes. The neighbor-joining tress was constructed by mega 3.1 program using kimura-two parameters as distance estimation and percent frequencies of the groupings were determined after 500 bootstrap evaluation.
Results: All the Colombian isolates analyzed were included within the genotype 2 and were closely related to linage 5 strains where the prototype VR2332 is found. The Colombian genotype 2 sequences revealed a nucleotide similarity of ORF5 among the isolates ranged from 91.2% to 99.8% and had a 57 - 60% of nucleotide similarities with Lelystad strain. These 55 Colombian isolates also shared a high nucleotide homology with the MLV RespPRRS vaccine strain which was used in our country about 15 years ago.
Conclusion: Some Colombian farms are affected by genotype II PRRSV and its spread seems to be very low. Important nucleotide variations were detected within ORF5 antigenic epitopes in some isolates collected from the same farm or the same geographic regions. This study also demonstrated that a low genetic variability exists among the circulating strains which may be explained by the low selective pressure because in our country the MLV was only used until 2004. It is concluded that circulating field strains may be vaccine virus derived strains.

Disclosure of Interest: None Declared

Keywords: Colombia, phylogenetic analysis, PRRS diversity, control

Viral and Viral Diseases
PRRS

PO-PW1-192

Epidemiological surveillance of PRRS in pig farms from Yucatán, Mexico.

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Introduction: The acquisition of replacement gilts represents the main risk for the introduction of the disease to negative farms, or in the case, for the introduction of new virus variants to positive farms. The possibility of boars transmitting PRRS is important even when the animal does not show signs of the disease after infection. **Objective:** To determine the health status of replacement gilts and boars used for natural mating or semen donors regarding the virus of PRRS in farms using different control/prevention measures for the disease.
Materials and Methods: Blood samples were obtained in order to extract serum from animals of 23 farms that produce their own gilt replacements, four samplings were carried out. Other 25 farms which use natural mating were also sampled. Sera were tested with the ELISA-herdChek® X3 PRRS, in order to determine the status of the animals regarding the disease. ELISA positive samples were tested with a Polymerase chain reaction procedure specific for PRRS virus, trying to find evidence of the actual virus. It is important to stress that in Yucatan there aren´t registers of PRRS samplings as in other parts of Mexico.
Results: Eight out of 25 (32%) farms with boars resulted positive to PRRS virus by serology. 36 sera out of 128 (28.13%) from boars, were positive in the ELISA X3. Fifteen15 out of 23 (65.22%) farms using self-replacement gilts were positive to PRRS virus while 625 sera out of 1414 (44.21%) obtained from replacements gilts were positive to the ELISA X3. Four quarintine areas with 155 animals were tested with no positive ELISA X3 results. All samples from boars (36) positive in the ELISA X3 were negative in the PCR. Six out of 635 ELISA X3 positive samples from replacement gilts were positive in the PCR., they belonged to two farms.
Conclusion: The ELISA X3 results indicate that both replacement gilts and boars have been exposed to PRRS virus (time of exposure was not determined). With respect to the PCR test, only two farms are certain to have the PRRS virus, because sera is not the best sample to detect the virus due to the short viraemia. Considering the results it is possible to conclude that at least two of the sampled farms have PRRS virus circulation. It is suggested that prevalence of PRRS is low. It is recommended to sequence the PCR amplifications to determine the virus strain present in the positive farms, so specific control measures like vaccination can be implemented.

Disclosure of Interest: None Declared

Keywords: Boars, gilt, PRRS

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-122

SEQUENCE ANALYSIS OF AN ITALIAN PRRSV-1, SUBTYPE 1 CAUSING SEVERE CLINICAL OUTCOME

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) is a devastating disease affecting the swine industry worldwide. Since the late 90's highly pathogenic (HP) isolates have emerged causing overt and severe clinical signs either in sows (Sow Abortion and Mortality Syndrome – SAMS) or in weaners-growers in North America, Asia and, more recently, in Europe. These isolates are characterized by high viral loads, high fever, severe general clinical signs and increased mortality. Moreover, they present a discontinuous aa deletion in the non-structural protein 2 (nsp2). In this study, the complete genome sequence of an Italian PRRSV-1 subtype 1 causing a severe clinical outbreak was analyzed.
Materials and Methods: In a farrow to wean 1500-sow herd, an increase of post-weaning mortality (peaking up to 50%) was associated with severe systemic and respiratory disease. PRRSV was demonstrated by PCR and isolated on PAMs. The isolate (PR-402014) was submitted to full genome sequence analysis. Total RNA was extracted and converted into first-strand cDNA. Double-stranded cDNA was synthesized and amplified simultaneously with random primers via whole genome amplification method. Shotgun DNA library was constructed from the amplified ds cDNA and subjected to massive parallel sequencing on Illumina MiSeq System at 2x300bp module following the manufacturer's instruction. The reads obtained was initially trimmed with quality filter and mapped to the reference genome. The mapped reads were assembled into consensus contig for a complete viral genome.
Results: The whole genome sequence of the isolate was obtained with a size of 14,678bp. The nucleotide sequence of the ORF5 showed 85.9% homology to Lelystad Virus. The isolates belong to the Italian cluster of PRRSV-1, subtype 1. A discontinuous deletion of 42nt and 366nt was identified in the nsp2 region, and a 6nt deletion was seen in the ORF4.
Conclusion: HP-PRRSVs are characterized by a deletion in the nsp2. However, this deletion is likely not the only factor responsible for differences in virulence, as nsp2 is the most divergent region in PRRSV. Also for this isolate, the sequence analysis showed a discontinuous deletion in nsp2 region, strengthening the hypothesis that this mutation may be important for and associated with an increased virulence of the isolate. The experimental infection of susceptible pigs with this isolate and further genetic and immunological analysis are ongoing.

Disclosure of Interest: None Declared

Keywords: HP-PRRSV, nsp2, whole genome sequence

Viral and Viral Diseases
PRRS

PO-PW1-146

Successful WT-PRRSV elimination in a 1500-sow farrow-to-finish system through mass vaccination and keep loading PRRSV negative gilts

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Introduction: PRRS has a significant economic impact on Chinese swine industry. Since high swine farm density and a lot of constrains to controlling PRRS, elimination wild type PRRSV (WT-PRRSV) is very hard in China. Some farmers used repopulation and depopulation successfully eliminated WT-PRRSV, but this method is very costly. We report here on the successful elimination of WT-PRRS virus from a 1500 sow farrow-to-finish production system easily to success.
Materials and Methods: In 2006 summer, a closed 1500 sow production system suffered highly pathogenic PRRS outbreak. Nursery mortality went up to 30% and caused abortion storm in the next 1 month. Then 3 commercial vaccines were chosen one by one to control PRRS, but mortality still moved between 15%>30%. Since March 2008, Ingelvac PRRS MLV was used to control PRRS, and executed as below: stop loading gilts for half year, gilts isolated at least 2 month and vaccinated twice before moved to breeding herd; breeding herd vaccinated 2 times at beginning apart 30days, then quarterly vaccination. Piglets vaccinated at 15 days. All the nursery and finishing sites were operated strictly all in/all out by site. Farm was just open to load PRRS negative gilts twice from 2008 to 2015 and continued using Ingelvac PRRS MLV for 7 years. Different age pigs' serum samples were collected for disease monitor 2 to 6 times every year. Serum samples collected from 2009 to 2015 is 280, 90, 31, 87, 110, 136 and 80 separately. All samples were individually tested on the IDEXX PRRS 2XR ELISA; then pooled 5:1, with the pools tested using a PCR and sequenced PRRSV positive samples at Nanjing agriculture university diagnostic laboratory. Local veterinary station collects 30 tonsil samples to test W-T PRRSV every half year.
Results: Both serum and tonsil samples from growing pig sites have tested W-T PRRSV negative by PRRS PCR and also not show PRRS clinical sign since 2014. W-T PRSSV positive rate within serum from 2009 to 2014 is 22.2%, 11.1%, 16.1%, 5.7% and 4.5%. W-T PRSSV positive rate within Tonsil from 2009 to 2014 is 66.7%, 41.7%, 16.7%, 8.3% and 8.3%. Meanwhile, wild type CSFV and PRV were also eliminated from 2013.
Conclusion: contiuted to use Ingelvac PRRS MLV and just load negative gilts twice from 2008 to 2015 is very important to this successful elimination. We can learn from this case continue using one effective PRRS MLV and loading negative gilts can successfully eliminate wild-type PRRSV.

Disclosure of Interest: None Declared

Keywords: None

Viral and Viral Diseases
PRRS

PO-PW1-164

Effect of Ingelvac® PRRS MLV on semen quality after boar vaccination

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Introduction: Vaccination is one of the effective methods to control boar from PRRSV infection in China. But, many people are hesitant to vaccinate boars because they worried vaccination could affect boar semen quality. The aim of this study was to evaluate the effect of a PRRS modified live vaccine (Ingelvac® PRRS MLV) on boar semen quality to provide a reference basis for PRRS boar vaccination.
Materials and Methods: The trial was carried out in a 2100-sow farrow-to-finish production system in China. It was a PRRSV positive stable farm. sows were massively vaccinated with Ingelvac® PRRS MLV three times per year and piglets were one shot at 14 days of age. boars were without vaccination. 13 adult boars with average weight 250~300 kg and 15 to 22 months of age were randomly divided into experimental group and control group; each group had 7 boars and 6 boars respectively. Each boar was in a single pen at the same closed and isolated house. House cleaning, disinfection and vaccination of other vaccines were implemented the same and according to the farm's procedures.7 boars in experimental group were vaccinated with Ingelvac® PRRS MLV at the same day. Semen collection was in accordance with the normal production schedule of the farm which was about one collection per week. Semen index of every boar (sperm quantity, sperm density, sperm motility and malformation rate) were recorded and compared before and after vaccination within one month.
Results: The statistical analysis of the recorded semen data of 7 experimental boars before and after PRRS vaccination, revealed no significant differences in semen collection capacity (P =0.8746), Sperm density (P =0.1347), sperm vitality (P =0.8744), while there was significant difference (P =0.0468) in sperm malformation rate, and the sperm deformity rate of boars before vaccination was higher than that of boars after vaccination. In addition, in the period of time after vaccination, there was no significant difference in 4 semen quality indexes between control and test groups. The P values of sperm quantity, sperm density, sperm motility and malformation rate were 0.093, 0.6597, 0.7966 and 0.5066.
Conclusion: This experiment showed vaccination of Ingelvac® PRRS MLV would not affect boar semen quality. An interesting finding was Ingelvac® PRRS MLV could provide some certain improvement in sperm malformation rate of experimental boars. Four weeks before the trial total 13 boars were collected blood sample to do PRRSV infection test, the only positive one was randomly assigned into the control group. We were not sure whether this was factor that affected the result, and we need to get more date to confirm this conclusion.

Disclosure of Interest: None Declared

Keywords: None

Viral and Viral Diseases
PRRS

PO-PW1-183

Phylogenetic analysis of ORF 7 sequences of Porcine Reproductive and Respiratory Syndrome Virus (PRRSv) in Mexico.

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Introduction: PRRS is the most important disease that affects swine industry, causing large economic losses. Several studies demonstrate that differences between virus strains can affect the kinetics of immune response. The high mutation rate of the virus causes changes in the virus antigenicity, compromising the effectiveness of diagnosis and vaccines. Despite this, little is known about variants in Mexico.
Materials and Methods: Sampling was conducted in prevalent PRRSv areas (nasal swabs). RNA extraction was performed with PureLink RNA/DNA extraction kit and RT-PCR with QIAGEN® OneStep RT-PCR kit. The products were placed in a 2% agarose gel and run to 90 volts for 50 minutes. Purification was done using the QIAquick® gel extraction kit and the products were sent to the Institute of Cell Physiology, UNAM for sequencing. Sequence analysis was performed with Chromas Pro to create consensus sequences, subsequently aligned using CloneManager Suite7 program with the American reference strain VR2332 (EF536003) and the European reference strain (M96262). Phylogenetic tree was constructed with an alignment of sequences reported in GenBank, including reference strains. The alignment was performed in the ClonManager Suite7 program with the overall DNA algorithm. Sequences were progressively aligned online using MAFFT V.7., the JModelTest analysis was used to elucidate the phylogenetic relationships with the criterion of maximum likelihood (ML). The algorithm was applied to determine the best substitution model, along with 1000 bootstrap replicates to provide statistical support.
Results: Separation of two major clades was observed. The lower groups are type 1, while the upper type 2 strains. Our isolates cluster with type 2; this clade is divided into two main branches. The bottom includes sequences from India, EE. UU., and China, as well as a sequence from Mexico. The upper branch is also divided into several sub branches. In one of them we find sequences of the isolates obtained in this study (triangle) as well as two field samples (CSI and 357). It is noteworthy that the A4 and A16 strains form a small branch slightly separated.
Conclusion: The clustering of the isolates with the reference strain may imply a restrain of variation due to the extensive use of the vaccine, however it does not imply that the vaccine protects effectively. Endemic strain identification is essential for the development of vaccine and diagnostic tests with specific targets towards circulating strains. Further analysis involving the identification of genetic variations in the complete genome of the virus is important to determine other regions involved in genetic variation.

Disclosure of Interest: None Declared

Keywords: Mexico, ORF7, PRRS

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-129

PRRS and Lawsonia intracellularis infections during the starter and grower period of finishing pigs reduce ADG and increase FCR

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Introduction: PRRS is directly and indirectly an important risk factor for the Porcine Respiratory Disease Complex influencing finishing pig performance. A lot is known from controlled experimental studies. However, studies under practical circumstances about the influences of various pathogens on finishing pig performance are scarce.
Materials and Methods: In total 815 pigs were individually followed from birth (December 2012-january 2013) until slaughter (June-July 2013). Pigs were identified by a chip containing an ear notch. Pigs were vaccinated against *Mycoplasma hyopneumoniae* (Mhyo) and PCV2. Out of these pigs, 116 pigs were randomly selected for longitudinal serological surveillance and were bled by jugular venipuncture and weighed at 9-(T1), 14- (T2), 18- (T3) and 22-weeks (T4) of age. At approximately 10 weeks of age at 20 kg, pigs were transported to the finishing farm. Carcass data of the pigs was collected in the abattoir. Serum was stored at -32°C until analysis. Serum samples T1 and T4 were analyzed for antibodies against PRRS, PCV2, Mhyo, Influenza (INF), *Actinobacillus pleuropneumoniae* (APP) and *L. intracellularis* (LAW) by the MSD R&D Service Laboratory (Boxmeer NL). After analyzing the T1 and T4 samples, it was decided to measure the T2 and T3 samples for PRRS, LAW and APP. An infection was defined by a change from negative towards positive in two consecutive periods based on the test specifications. Data was analyzed with SAS using generalized linear mixed models (Proc Glimmix).
Results: Infections occurred predominantly with the pathogens APP (93.2%), Law (87.9%), and PRRS (76.7%). Infections with the other pathogens was of a lesser extend (Mhyo 1.7%; INF 21.6%, PCV2 14.4%). Technical performance measured by ADG was mainly affected by infections with PRRS, showing a significant ($p<0.01$) reduction of ADG in the starter period (T1-T2; n = 26; 59 gram ADG) and the grower period (T2-T3; n = 39; 64 gram ADG). In the finishing period (between T3 and T4) only 3 animals were infected with PRRS. PRRS infections during the starter and grower period resulted in a significantly lower carcass weight at slaughter of 3.4 and 4,. kg respectively ($p=<0.05$). PRRS infections in the starter period resulted in a higher FCR (0.15; $p<0.01$). Infections with LAW during the grower period resulted in a significant reduction in ADG of 39 grams ($p<0.05$).
Conclusion: PRRS and LAW seroconversion reduced ADG with ~60 grams/day and increased FCR with 0.15. This study shows that the effect on ADG, due to a PRRS infection, does not differ between the starter and the grower period.

Disclosure of Interest: None Declared

Keywords: None

Viral and Viral Diseases
PRRS

PO-PW1-115

Evaluation of novel inactivation method of PRRSV for vaccine production

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) is an economically important animal virus that causes reproductive failure and respiratory track illness in pigs. Current inactivated vaccines have low efficacy and/or complicated time-consuming production procedure requiring application of hazardous reactants such as formaldehyde or binary ethylenimine. Here, we studied the possibility of hydrogen peroxide (H_2O_2) as a suitable alternative for inactivated vaccine preparation.
Materials and Methods: Inactivation procedure was performed by incubation of North American PRRSV strain NADC-20 solution with different concentrations of H_2O_2 in various environmental conditions and evaluated its virucidal efficacy at two time points. *In-vitro* studies with MARC-145 cells, inoculated with inactivated viral solutions, demonstrate successful inactivation of virus with the absence of cytopathic effect even at very low H_2O_2 concentration of 0.5% after 1 hour of inactivation procedure.
Results: Cell proliferation assay was performed to confirm the results from microscopic observations. It was also found that catalase from bovine liver is more suitable reagent for removal of residual H_2O_2 from viral solution than iron (III) chloride, because it maintains neutral pH and it is biocompatible with living cells.We also observed that H_2O_2-inactivated PRRSV have similar immunogenicity pattern as live virus in a western blot analysis using serum from pigs infected with wildtype PRRS virus.
Conclusion: Our studies suggest that H_2O_2-inactivated virus can be a promising candidate for further *in-vivo* investigation to confirm its efficacy in creating adequate immune protection. Efficacy study of PRRS vaccine prepared with this new inactivation method is ongoing and will be reported at this meeting.

Disclosure of Interest: None Declared

Keywords: PRRS, inactivation, vaccine, efficacy

Viral and Viral Diseases
PRRS

PO-PW1-200

Field trial: Stabilizing PRRSv with Tilmovet® and a live attenuated vaccine

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Introduction: This abstract describes a field experience with a Tilmovet® program and a live attenuated vaccine (EU strain, Porcilis® PRSS) to stabilize PRRSV in the sow herd and its effect on the progeny.
Materials and Methods: A Belgian 1200 sow farm positive for *Mycoplasma* was confronted, despite a high biosecurity level (4 week batch-farrowing and all-in-all-out), with chronic respiratory problems in the nursery and fattening units and consequently disappointing zootechnical results. Serology performed in October 2014, revealed high and variable antibody titers for PRRSv in sows and piglets. The mean S/P ratio in the sow herd was 2.29 and 55% of the values were above 2 (= indicative for an unstable sow herd), despite intensive vaccination (day 60: live attenuated vaccine, EU strain Porcilis® PRRS and day 90: inactivated vaccine, Progressis® PRRS). As expected, because of the unstable situation in the sow herd, high and variable PRRSv S/P ratios were also observed in the nursery (at 10 weeks of age: mean: 1.71 with a range of 1.09-3.02). The veterinary practitioner considered PRRSv as one of the main involved pathogens. This is why an alternative approach, combining Tilmovet® (tilmicosin 200 mg/g) and an adjusted vaccination scheme, was implemented. All sows were vaccinated (EU attenuated strain, Porcilis® PRRS) once at 60 days of pregnancy and treated with Tilmovet® (10 mg tilmicosin per kg bodyweight) from 7 days before until 7 days after farrowing. Previous studies already illustrated the effect of Tilmovet® on PRRSv. Gilts were vaccinated twice (attenuated vaccine, EU strain, Porcilis® PRRS) during rearing and received Tilmovet® (10 mg tilmicosin per kg bodyweight) during 14 days before insemination. The piglets were medicated with Tilmovet® at 16 mg tilmicosin per kg bodyweight for 14 days after weaning.
Results: Six months after implementing the new approach, new blood samples were taken. The S/P ratios in the sow herd decreased to a mean of respectively 1.57, with 90% of the samples below 2, which is indicative for a stable sow herd in case of vaccination. All of the tested piglets of 15 weeks of age, were serological negative for PRRSv. The weaned and 10 old week piglets showed average S/P ratios of respectively 1.43 and 0.49, most likely maternal antibodies. Also the zootechnical expectations were met again.
Conclusion: This illustrates again that the main objective to control PRRSv at herd level is to stabilize the sow herd. By doing this also the infection pressure in the progeny will decrease. Next to biosecurity and management also vaccination and Tilmovet® can be a valuable tool to control PRRSv.

Disclosure of Interest: None Declared

Keywords: tilmicosin PRRS vaccination

Viral and Viral Diseases
PRRS

PO-PW1-140

Serum-derived exosomes from non-viremic animals previously exposed to the PRRSV contain immunogenic viral proteins

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Introduction: PRRSV is one of the most important diseases of veterinary interest with a significant economic burden worldwide and limitations in vaccinology; thus, new alternatives to conventional approaches are desperately needed to control and eventually eradicating it. Exosomes are 30-100 nm vesicles of endocytic origin originally described as a garbage-disposable mechanism of reticulocytes. Remarkably, antigens associated to exosomes are capable of eliciting specific and protective immune responses, albeit variably, in cancer and infectious diseases. Here we describe the isolation, molecular composition and immunogenicity of serum-derived exosomes from naïve pigs and from pigs previously infected with PRRSV.
Materials and Methods: Sera were obtained from naïve animals pertaining to a certified farm never reporting infections by PRRSV, from viremic animals, and from animals previously infected already free of viruses. Exosomes were isolated through size exclusion chromatography and characterized by Bradford assay, Flow cytometry, Nanoparticle tracking analysis (NTA), SDS-PAGE, Cryo-TEM and LC-MS/MS. Later, combined Sandwich- indirect ELISA was used to evaluate the capacity of PRRSV immune and naïve sera to recognize exosomes derived from non-viremic and naïve animals.
Results: Exosome-enriched fractions from naïve and natural infected animals contained classical exosomal markers (CD63 and CD81) and high concentrations of particles in the size-range of exosomes as detected by NTA and cryo-TEM analyses. Moreover, immune sera from pigs previously exposed to PRRSV, specifically reacted against non-viremic sera derived exosomes in a dose-dependent manner when tested by ELISA, which was not seen when naïve sera was used. Then nanoLC-MS/MS was used to identify viral antigens associated to exosomes. PRRSV-proteins were detected in samples from viremic animals and from animals previously infected already free of viruses.
Conclusion: To the best of our knowledge this is the first molecular charaterization of serum-derived exosomes from naïve pigs and pigs actively or previously infected with PRRSV. Their immunological properties and viral protein content warrant further vaccine studies with these nanovesicles as classical approaches had shown limited effectiveness.

Disclosure of Interest: None Declared

Keywords: immunogenicity, PRRSV, Serum-derived exosomes

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-187

Effect of 4 commercial PRRS vaccine on the CSFV vaccine antibody response

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Introduction: PRRS and CSFV vaccine are both widely used in China. Most of time they will be vaccinated on pigltes apart 2 weeks. Studies have shown that vaccinating CSFV vaccine 2 days after PRRS vaccination will reduce CSFV immunity response.From our farm routine antibody monitor program, we found CSFV antibody positive rate always low(<30%), so we designed to investigate if vaccinated piglets CSFV vaccine 2 weeks after PRRS vaccination still influence CSFV antibody response.
Materials and Methods: 164 piglets from 15 littles were randomly separated into 5 groups. Group A (n=32) vaccinated local "classic strain" PRRS vaccine, Group B (n=30) vaccinated another local "classic strain" PRRS vaccine, Group C(n=33) vaccinated highly pathogenic strain PRRS vaccine, Group D(n=35) vaccinated Ingelvac PRRS MLV, Group E (n=34) were control. Group A, B, C, D vaccinated PRRS vaccine at 15 days, and C-strain CSFV vaccinate at 30 days; control group just vaccinated CSFV vaccine at 30 days. After wean, every group were separated into different pens and not mixed. All the management and other vaccination program for these 5 groups are all the same. serum samples were collected from all piglets 35 days after CSFV vaccinattion. Serum concentration of CSFV antibody was determined by IDEXX ELISA kit (IDEXX CSF Sero). The cut off value of the CSF ELISA is 0.4 in blocking rate.
Results: CSFV antibody positive rate of group A is 25%, group B is 20%, group C is 21.1%, Group D is 82.9%, control group is 85.3%. P value between Group D and Group A is <0.0001, Group D and Group B is <0.0001, Group D and Group C is <0.0001, Group D and control is 0.8264. 3. From this study, we found local commercial PRRS vaccine will significant reduce CSF vaccine antibody response even 2 weeks apart vaccination, meanwhile, Ingelvac PRRS MLV is safe to use and not influencing the CSF antibody response apart 2 weeks vaccination.
Conclusion: Some farms get confused to low CSFV antibody positive rate after vaccination even choose high quality commercial CSFV vaccine. From this study we found some PRRS vaccine will reduce CSFV vaccine antibody response, farmers should also pay attention to PRRS vaccine's role on CSFV antibody response.

Disclosure of Interest: None Declared

Keywords: None

Viral and Viral Diseases
PRRS

PO-PW1-114

Porcine Reproductive and Respiratory Syndrome: effect of PRRSV-1 strains of differing virulence in bone marrow of piglets

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Introduction: Many studies have demonstrated the remarkable phenotypic and genetic diversity between strains and within subtypes of porcine reproductive and respiratory syndrome viruses (PRRSV). Experimental infections have demonstrated replication of PRRSV strains, including of highly pathogenic strains (HP-PRRSV) in primary lymphoid organs such as the thymus. However, studies of the bone marrow are scarce but necessary to help elucidate the mechanisms of immunopathology and immunomodulation by PRRSV strains of differing virulence. In this study we detected viral RNA and PRRSV-positive cells within the bone marrow of animals experimentally infected with both low virulent Lelystad (LV) and 215-06 PRRSV-1 strains and with the HP-PRRSV SU1-bel strain.
Materials and Methods: Fifty-four, 5-week-old, male piglets were inoculated by intranasal route with sterile medium (control group) or with one of three different PRRSV-1 strains ($10^{5.0}$ $TCID_{50}$): Lelystad virus strain (LV), the British field strain 215-06 and the HP-PRRSV Eastern European strain SU1-bel. Animals were clinically monitored and euthanised at 3, 7 and 35 days post infection (dpi). Samples from bone marrow were routinely processed for histopathological and immunohistochemical studies by using specific antibodies against PRRSV, TUNEL, cleaved Caspase 3 (cCasp3), IL-1α, IL-6 and TNF-α. Total RNA was extracted from the bone marrow and RT-qPCR was performed to analyse viral load.
Results: Whereas the highest RNA levels were detected in pigs experimentally infected with LV, PRRSV-infected cells were only detected in one animal infected with SU1-bel strain. On 3 dpi, a decrease in the proportion of haematopoietic tissue and number of erythroid cells in all infected groups was associated with an increase in TUNEL or cCasp3 labelling and higher counts of myeloid cells compared to control animals. The expression of IL-1α and IL-6 was elevated at the beginning of the infection in all infected animals. The expression of TNF-α was increased at the end of the study in all infected groups with respect to control.
Conclusion: Our results demonstrate that viral load in the bone marrow is independent of *in situ* PRRSV replication and associated to circulating virus. In addition, different PRRSV-1 strains induce, presummably by indirect mechanisms and independently of the virulence, moderate and transient hypoplasia of erythroid cells and myeloid cell hyperplasia in the bone marrow of experimentally infected piglets at early stages of infection. In addition, these changes are paralleled by a peak in the local expression of IL-1α, IL-6 and TNF-α in all infected animals.

Disclosure of Interest: None Declared

Keywords: bone marrow, PRRSV-1, virulence

Viral and Viral Diseases
PRRS

PO-PW1-199

Dynamic of PRRS virus in farms with different strategies of control, in Yucatan Mexico.

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Introduction: It is possible that the PRRS virus is presented like asymptomatic. As in most pig production regions in the world, many farms in Mexico are positive to PRRS virus, however in the Southeast region there is a little clinical evidence of the disease. The objective was to understand the dynamic of PRRS virus in farms that are employing different strategies to control the diseases.
Materials and Methods: Ten farms with serological history were used, four were considered negative and six positive. In three of the positive farms, the diseases control is employing MLV vaccine. The fourth farm produces its own replacement by ELISA X3 test, checks that the 100% is seropositive (SP> 0.4) and before introduce to herd and the last two farms do not take any strategies of control. In the negative farms are done ELISA X3 test, to the incoming animals. In the positive farms were obtained 28 blood samples in sows of different parities (14 at the beginning and 14 to end of the study), 120 blood samples from piglets of different ages and 48 oral fluid (OF) samples (24 in maternity and 24 in wean area). By other hand in the negative farms were obtained a total of 270 blood samples (135 at the beginning and 135 to the end) in sows of different parities. Also 120 blood samples of piglets to different ages and 144 OF samples (72 in maternity and 72 in weaning) were taken. Sera samples were analyzed with ELISA-HerdChek® PRRS X3 test and Real Time - PCR; OF samples with ELISA OF and RT-PCR.
Results: In negative farms, two showed 100% negative samples and two positive. Five farms results with ELISA X3, inconsistent with their initial status. RT-PCR test was performed to a total of 1692 sera of five farms with inconsistent in ELISA X3, ELISA and RT-PCR in OF results to their initial status. Four farms belonged to negatives: two were 100% negative to RT-PCR and two positives. The fifth farm with positive initial status result 100% negative to ELISA X3.The farms with positive initial status presented at least one sample positive to ELISA OF. In OF PCR, three farms showed at least one sample positive and three were 100% negative samples. The farms with negative initial status showed at least one positive sample to both test.
Conclusion: Based on the results, two farms have a different status despite having no clinical signs consistent with PRRS. It was confirmed that the oral fluids samples are a good alternative for surveillance studies in fattening pigs. PCR results, suggest that the circulation of the PRRS virus in most farms is low. It is necessary to perform more than one test for disease surveillance in the farm.

Disclosure of Interest: None Declared

Keywords: Oral fluids, PRRS, RT-PCR

Viral and Viral Diseases
PRRS

PO-PW1-162

Assessing the role of PRRSV minor glycoproteins in the induction of a protective immune response

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Introduction: The role of PRRSV minor glycoproteins in the induction of a protective immune response is an area of PRRSV research that may have an impact on vaccine development. GP2, GP3, and GP4 form a trimer on the surface of the virion and it has been demonstrated that GP2 and GP4 interact with the CD163 receptor on host cells, and because of the trimer formation, GP3 may be interacting with the receptor as well. Previous work by many laboratories has shown minimal cross-protection between Type I and Type II PRRSV. With this knowledge, GP2, GP3, and GP4 of a Type II infectious clone were cloned into a Type I infectious clone, which serves as a vector for the proteins. The chimeric virus is being used to elucidate the contribution of the minor glycoproteins to a protective immune response in swine.
Materials and Methods: The GP2, GP3, and GP4 of a Type II infectious clone (FL12) were PCR amplified and then cloned into a Type I infectious clone (SD0108) in place of the corresponding Type I proteins. Infectious chimeric virus (SDFL24) was recovered and serum-virus neutralization assays were performed in MARC-145 cells to evaluate the sensitivity of the SDFL24 to FL12-infected, convalescent sera. An animal study to evaluate protection *in vivo* is currently ongoing.
Results: The SD0108 construct containing GP2, GP3, and GP4 of FL12 is fully infectious. The chimeric virus SDFL24 grew well in MARC-145 cells to a titer of $10^{5.75}$ $TCID_{50}$/ml. While the parental strain SD0108 is not sensitive to neutralization by FL12 convalescent sera (mean antibody titer of >1:4), the chimeric SDFL24 is sensitive to neutralization by FL12 convalescent sera with a mean titer of 1:32. However, SDFL24 is less sensitive to neutralization when compared to FL12, which has a mean titer of 1:200. An animal experiment to evaluate the role of the proteins in a protective immune response is ongoing. Sera from infected animals will be used to characterize the neutralizing antibody response against parental and heterologous PRRSV strains. PBMCs will also be harvested and the cellular immune response will be evaluated. Protection after challenge will be measured by viremia and viral load in tissue.
Conclusion: Swapping the minor glycoproteins of Type II into a Type I backbone did not alter the replication of the virus. The chimeric virus containing only GP2, GP3, and GP4 of Type II is sensitive to neutralization by Type II convalescent sera, indicating that GP2, GP3, and GP4 are important for neutralization. This study allows us to weigh the relative contribution of GP2, GP3, and GP4 to overall neutralization. The protective capabilities of the chimeric virus are being evaluated in an animal experiment.

Disclosure of Interest: None Declared

Keywords: Minor glycoproteins

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-194

Relationship between vasculitis, endometrial inflammation and cell death at maternal-fetal interface of type 2 PRRSV infected pregnant gilts

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) is a well-known cause of reproductive failure worldwide. It is known that PRRSV can induce apoptosis under in-vitro conditions, and is significantly associated with cell the occurrence of cell death in multiple infected tissues in-vivo. The mechanism of cell death occurring at the maternal-fetal interface during PRRSV infection, and the relationship of cell death to the cytopathic effect of the PRRSV and other pathogenic factors remain unclear.
The objective of this study was to determine if the PRRSV induced endometrial inflammation and vasculitis along with presence of virus are associated with the occurrence of cell death at the maternal-fetal interface
Materials and Methods: A total of 114 PRRSV-naïve high-health pregnant gilts were inoculated with a type 2 PRRSV (NVSL 97-7895) and 19 negative control gilts were sham inoculated on gestation day 85±1. At 21days post inoculation (DPI), dams and their litters were humanely euthanized for necropsy examination. The adjacent uterus and placenta was graded based on the percentage of affected endometrial tissue and the total number of inflammatory cells present. The degree of vasculitis was assessed based on its distribution and severity within the endometrium. The same tissues (n=248) were also examined by TUNEL assay to detect apoptosis. Numbers of apoptotic cells per 1mm^2 area of the endometrium and fetal placenta were determined by Image ProPlus software.
Results: Moderate lymphohistiocytic endometritis was in 60.48% (150/248) of the tissue sections. Grade 1 vasculitis was most common in 52.82% (131/248) of endometrial vasculature. However, statistical analysis revealed that only distribution and severity of vasculitis in combination with viral load have a significant positive association with the numbers of TUNEL positive cells at the maternal-fetal interface ($P<0.05$, $P<0.001$, $P<0.001$, respectively; linear mixed model, clustering by gilt). Apoptosis was not significantly associated with the severity of inflammation affecting lamina propria, uterine gland and interdigitation areas of maternal-fetal interface.
Conclusion: In the related work from this project, there was no relationship found between the viral loads and the amount of endometrial inflammation within the uterus.
While the inflammation score was not associated with apoptosis, the findings from this project demonstrate a connection between vascular lesions in the pathogenesis of cell death at the maternal-fetal interface during type 2 PRRSV infection of pregnant gilts at 21 DPI. Further work examining this relationship at earlier time points in the infection process are underway.

Disclosure of Interest: None Declared

Keywords: apoptosis, Pathogenesis, PRRS

Viral and Viral Diseases
PRRS

PO-PW1-176

Activation of interferon regulatory factor 3 upregulates endogenous SAMHD1 expression is vital for antiviral innate immunity

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Introduction: The sterile alpha motif and HD domain 1 (SAMHD1) protein has been identified as a novel innate immunity restriction factor that inhibits HIV-1 infection in myeloid cells, and is a type I interferon (IFN) inducible restriction factor. Previous study showed that overexpression of porcine SAMHD1 efficiently blocked highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) replication in MARC-145 cells, and SAMHD1 is activated in target cells (porcine alveolar macrophages, PAMs) after infection of PRRSV. The underlying mechanisms of SAMHD1 transcriptional regulation by virus infection remain elusive.
Materials and Methods: Here, we used porcine reproductive and respiratory syndrome virus (PRRSV) and Newcastle disease virus (NDV) infection as models and show that inducing SAMHD1 upregulation is part of an early intrinsic immune response via TLR3 and RIG-I/MDA5 agonists that ultimately induce the nuclear translocation of the interferon regulation factor 3 (IRF3) protein.
Results: IRF3 plays a major role in upregulating endogenous SAMHD1 expression in a mechanism that is independent of the classical IFN-induced JAK-STAT pathway. Both overexpression and activation of IRF3 enhanced the SAMHD1 promoter luciferase activity and IRF3 activation was necessary for upregulating SAMHD1 expression in type I IFN cascade. We also show that the SAMHD1 promoter is a direct target of IRF3 and an IRF3 binding site is sufficient to render this promoter responsive to stimulation.
Conclusion: Collectively, these findings indicate that upregulation of endogenous SAMHD1 expression is connected to the phosphorylation and nuclear translocation of IRF3 and we suggest that type I IFN induction and induced SAMHD1 expression are coordinated.

Disclosure of Interest: None Declared

Keywords: innate immunity, PRRSV, SAMHD1

Viral and Viral Diseases
PRRS

PO-PW1-181

The role of macrophages and the inhibition of highly pathogenic PRRS virus replication in pigs fed tylvalosin tartrate-medicated feed

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Introduction: An atypical and highly pathogenic porcine reproductive and respiratory syndrome (HP-PRRS) variant has occurred in China and Southeast Asia. This disease was characterized by a high fever of above 41˚C, anorexia, red discoloration of the ears (blue ear) and high mortality in pigs of all ages. Recently, it has reported that macrolide antibiotics may have an anti-viral effect on PRRSV. We previously demonstrated that the macrolide antibiotic, tylvalosin tartrate, had anti-viral activity against HP-PRRSV replication *in vitro*. In the present study, the macrolide antibiotic was tested for anti-viral activity against HP-PRRSV replication and for the role of macrophages *in vivo*.
Materials and Methods: Fifteen 4-week-old specific pathogen-free pigs were used in the experimental infection. Six pigs (group 1) and 3 pigs (group 3) were fed with 200 ppm of Aivlosin® plus 10 (1% premix of tylvalosin tartrate, ECO Animal Health Inc.) a day per pig for one week before the viral inoculation and during the experiment period. Group 1 and another 6 pigs (group 2) were intranasally inoculated with 10^5 $TCID_{50}$/pig Vietnamese HP-PRRSV isolate 2010 (100186-614 strain), while group 3 was used as uninfected control All pigs were monitored daily for clinical signs. Blood and oral fluids were sequentially collected until 12 days post-inoculation (dpi). When all pigs were necropsied at 12 dpi, gross findings were assessed and a bacterial examination was carried out. Viral RNA in the serum and tissues were measured by quantitative real time RT-PCR. The proportion of macrophages in PBMC was analyzed by flow cytometry.
Results: All pigs in groups 1 and 2 exhibited high fever, anorexia and dyspnea. During the experiment period, only one pig in group 2 died at 11 dpi. Moreover, one pig in each group 1 and 2 were moribund at 12 dpi and *E.coli* was isolated from these pigs at necropsy. Hemorrhages and consolidation of lung and blood spots in kidney were observed. Other pigs in infected group 1 and 2 had pneumonia and enlargement of various lymph nodes. The amount of PRRSV RNA in serum, oral fluid and tissues in group 1 was significant less than those of group 2 and the percentage of macrophages in PBMC of group 1 was lower than that in group 2. No clinical signs and lesions were observed in control animals (Group 3).
Conclusion: The replication of HP-PRRSV and the number of macrophages was reduced in pigs fed with Aivlosin®, tylvalosin tartrate, compared with the inoculated animals without untreatment. Like the result *in vitro*, the replication of HP-PRRSV might be inhibited by the antiviral activity of macrophages having improved, of pigs fed with tylvalosin tartrate.

Disclosure of Interest: None Declared

Keywords: Macrophage, Porcine reproductive and respiratory syndrome virus (PRRSV), Tylvalosin tartrate

Viral and Viral Diseases
PRRS

PO-PW1-190

Field Efficacy of New PRRS Vaccine Against Local Thai PRRS Strain

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Introduction: Porcine Reproductive and Respiratory Syndrome (PRRSV) was first isolated in Thailand in 1996 from suckling and nursery pigs. A modified live PRRSV vaccine is an efficient and effective tool to decrease the mortality rate and increase nursery and growing-finishing pig performance. The PRRSV modified live virus vaccine belonging to the US genotype was registered and its use became common practice in Thai commercial swine herds in 2007. The objective of this field study was to compare the efficacy of two commercial PRRS vaccines, the Ingelvac PRRS® MLV vaccine and the Fostera PRRS® vaccine, in nursery pigs against local PRRS strain in Thailand.
Materials and Methods: A total of 40 piglets from four parity sows were randomized by size and weight, were divided into two groups, T01 and T02 with 20 piglets each. Both groups were vaccinated at 2 weeks of age. T01 pigs were injected with the Ingelvac PRRS® MLV while the T02 pigs were vaccinated with the Fostera PRRS®. Blood samples were collected on days 0, 28, 57 and 98 post-vaccination. Moreover, the blood samples were kept in plain tubes and allowed to clot at room temperature. After that, the sera were separated and stored at ≤ -20 °C until analysis. All samples were checked for PRRSV antigen in the serum and ELISA titer using the Reverse Transcription Polymerase Chain Reaction (RT-PCR) and IDEEX PRRS 3X ELISA Antibody tests, respectively.
Results: All pigs in group T01 were found to have negative PRRSv antigen results on days 0, 28 and 98, respectively, these results were similar to the pigs in group T02. In addition, group T01 showed twelve of twenty samples positive (60%), while group T02 had only two of seventeen samples showing a positive result (11.8%) on day 57. In addition, pigs in group T01 had mean s/p ratios of; 2.359, 1.672, 1.0975 and 1.887 on days 0, 28, 57 and 98, respectively. Group T02 showed mean s/p ratios of; 1.799, 0.941, 0.762 and 1.017 on days 0, 28, 57 and 98 respectively. On day 28 post vaccination, three pigs in group T02 were sero-negative (negative is an s/p ratio < 0.4). However, some pigs in both groups were found to have a sero-negative result; three pigs in group T01 and five pigs in group T02 on day 57. On the last blood collection day, group T01 did not have a sero-negative result, while group T02 did show a sero-negative result for three samples.
Conclusion: PRRS vaccination was effective at reducing viremia, which the RT-PCR found that all pigs were negative at Day 0, and the mean serum s/p ratio of anti-PRRS antibody titers were high. In conclusion, the Fostera PRRS® vaccine had a more positive effect on reducing the viremia than the Ingelvac PRRS® MLV vaccine.

Disclosure of Interest: None Declared

Keywords: Immune response, PRRS vaccine, , Viremia

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-186

Assessment of the in-use stability of the PRRS virus of two different commercial PRRS MLV after reconstitution with PPV and SE vaccines

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Introduction: Nowadays, many vaccination programs have to be implemented in sows. Firstly, reduction of injections by administering vaccines simultaneously can improve both animal welfare and the farmer's labour efficiency. Secondly, these combinations must be safe and effective, the European Medicines Agency (EMA) being the institution responsible for guaranteeing the efficacy of these mixtures. The purpose of this study was to assess the in-use stability of the UNISTRAIN® PRRS vaccine after it has been reconstituted with ERYSENG® PARVO in comparison with the in-use stability of another European commercial PRRS MLV reconstituted with the SE and PPV vaccine from the same manufacturer.
Materials and Methods: A freeze-dried tablet of 25 doses of UNISTRAIN® PRRS was reconstituted with 50 ml of ERYSENG® PARVO (group A) and a freeze-dried tablet of a European commercial MLV vaccine was reconstituted with 50 ml of the SE and PPV vaccine from the same manufacturer (group B). A freeze-dried tablet of the same batch of UNISTRAIN® PRRS was reconstituted with 50 ml of its solvent (group C) and a freeze-dried table of the same batch of a European commercial PRRS MLV was reconstituted with 50 ml of its adjuvant (group D). Group C and D were used as controls. The titre of the PRRS virus from the different mixed vaccines was assessed at T = 0, 1, 2, 3 and 4 hours after reconstitution and they were kept at room temperature until the end of the study. The virus was titered by measuring its cytopathic effect in the MARC 145 cell line. According to the SPC for each of the products, the minimum cell culture infection dose (MCCID) of UNISTRAIN® PRRS is $10^{3.5}$ $CCID_{50}$ and for the other European commercial MLV vaccine it is 10^4 $CCID_{50}$.
Results: The results showed that in group A, the PRRS virus remained stable for 2 hours after the reconstitution. From 2 hours onwards, the virus titre started to decline but remained above the MCCID until the end of the study. In group B, an important drop in the PRRS virus titre was observed less than 1 hour after reconstitution and led to results clearly below the MCCID (10^4 $CCID_{50}$/dose). In the control groups for both vaccines, the PRRS virus remained above the MCCID until the end of the study.
Conclusion: The most important factor involved in keeping the registration of the combined use of an MLV PRRS vaccine and SE and PPV vaccine is to ensure that the PRRS virus is kept alive after they are mixed together. These results conclude that the viability of the PRRS virus after mixing UNISTRAIN® PRRS and ERYSENG® PARVO can be guaranteed for 2 hours after reconstitution.

Disclosure of Interest: None Declared

Keywords: None

Viral and Viral Diseases
PRRS

PO-PW1-197

Introduction of replacement gilts to PRRS-positive sow herds

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) is an important disease, which creates problems in the swine industry worldwide, including Denmark. After infection or vaccination with a modified-live vaccine (MLV) against PRRSV, the pigs will contract a prolonged viremia, and will thus be able to transmit virus to PRRS-naïve pigs. Danish recommendations for PRRS-positive farms are to introduce replacement gilts for a quarantine period of 12 weeks after exposure to a wildtype virus or vaccination with MLV. In some herds it is practical impossible to manage a quarantine for 12 weeks.
The objective of the present study was to investigate the impact of PRRSV MLV and quarantine facilities on the PRRSV status of replacement gilts at first mating. Furthermore, the study aimed to look at antibody levels, relative to the time from vaccination with PRRS MLV, and the age of the animals when vaccinated.
Materials and Methods: The study was a cross-sectional study and included 69 PRRS-positive sow herds. Five blood samples from replacement gilts were taken at each farm just before mating. The samples were analysed for PRRSV by RT-qPCR, ELISA and IPMA. A questionnaire regarding information about gilt recruitment strategy, vaccination strategy etc, were completed for each herd.
Results: Based on the analysis of blood samples, each group of gilts on each farm were defined as 'stable' (n=63) or 'unstable' (n=6). Being stable was defined by being negative by RT-qPCR and positive by ELISA. The study found no viremic gilts by RT-qPCR (stable), but found 6 farms with all five gilts being seronegative(unstable).
There was no significant difference (α=0.05) between the stable and unstable groups regarding the use of quarantine, duration of quarantine, gilt recruitment strategy, number of suppliers, and number of deliveries of replacement gilts. There was a strong tendency towards the use of quarantine resulting in stable gilts. Furthermore, no significant relation between age when vaccinated and the level of antibodies were found.
Conclusion: The study showed that all replacement gilts were PRRS virus negative at the time of first mating which were surprising especially in herds with no quarantine facilities. The explanation is probably that the estimated time from vaccination to insemination (when blood samples were taken) was 18 weeks on average. Six (8.7%) herds had ELISA-negative replacement gilts, meaning the gilts were not immunised against PRRSV at first insemination despite being vaccinated. These unstable gilts are at risk of being infected with PRRSV during gestation and by that contribute to the transmission of PRRSV within the herd.

Disclosure of Interest: None Declared

Keywords: Gilt acclimation, PRRSV control, PRRSV Live Vaccine

Viral and Viral Diseases
PRRS

PO-PW1-154

Cross-sectional study on risk factors for Porcine Reproductive & Respiratory Syndrome (PRRS) sow herd instability in German breeding herds

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) is among the diseases with the highest economic impact in pig production. Today, different control options are available at farm level including close & rollover and test & removal. These two options request herd stability in terms of virus transmission from dams to their offspring (Holtkamp et al. 2010, JSHAP). Therefore, appropriate assessment of the stability and potential risk factors influencing stability at farm level are important. The aim of this study was to investigate associations between different farm and management characteristics and herd stability in breeding herds in Germany.
Materials and Methods: In a cross-sectional study, 120 breeding herds in Germany, where nearly 100% of herds are endemically infected with PRRS virus (PRRSv), were investigated and different production and management parameters recorded in a questionnaire. To assess herd stability, blood was sampled from 30 suckling pigs out of 10 litters and examined for PRRSv by real-time PCR (Tetracore Inc., Rockville, USA). If all samples from a herd were negative, it was classified as stable, if at least one sample was positive, it was classified as unstable. All variables were tested for univariable associations with the herd status and those with a p-value ≤ 0.1 retained for multivariable testing. After check for multicollinearity and step-wise backward selection, the final logistic regression model contained four variables that were significantly associated with herd status.
Results: Herds working with a suckling period > 21 days had an odds ratio (OR) of 0.23 for being instable, compared to herds with a suckling period of ≤ 21 days (confidence interval (CI): 0.05-0.88). Moreover, every additional meter distance between the carcass bin for dead pigs and the actual sow barn, decreased the chance of being unstable (OR: 0.99, CI: 0.98-0.99). Herds with ≥ 2 pig herds in their vicinity (1000 m radius) had a 9.91-fold higher chance of being unstable than herds with maximum one other herd in their vicinity (CI: 2.35-60.8). Finally, the OR of being instable for herds with external employees was 4.52, compared to herds without.
Conclusion: The influence of the proximity of neighbouring herds on the sow herd stability is indicating a frequent airborne transmission of PRRSv, which has been described elsewhere. However, the frequency of this event is widely unknown. The protective effect of a longer suckling period is in contrast to the idea of reducing the risk of vertical virus transmission by keeping this period, i.e. time under risk, as short as possible. The results indicate that PRRSv transmission from the dam to their offspring is not increasing over time.

Disclosure of Interest: None Declared

Keywords: Epidemiology, herd stability, PRRS

Viral and Viral Diseases
PRRS

PO-PW1-203

A PRRSV vaccine based on the SAVE approach is attenuated and effective in protecting pigs against homologous virus challenge

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Introduction: Current PRRSV vaccines are often minimally protective. Rapid production of attenuated farm-specific vaccines is a feasible alternative to utilizing commercial vaccines. Recently the synthetic attenuated virus engineering (SAVE) approach was utilized to rapidly attenuate the wild-type PRRSV isolate VR2385. Specifically, the codon-pairs of the major envelope GP5 gene of PRRSV were rapidly de-optimized through a computer algorithm which resulted in a modified GP5 nucleotide sequence while at the same time retaining the original amino acid sequence. The resulting virus was designated SAVE5. The objective of this study was to determine the efficacy of SAVE5 to reduce or prevent PRRSV-associated clinical signs, lesions and viremia following experimental challenge with the homologous virulent parental strain.
Materials and Methods: Four groups of 9-10 three-week-old pigs were utilized. At day 0, two groups were vaccinated with the SAVE5 virus which is an attenuated version of PRRSV strain VR2385, while the two other groups were sham-vaccinated with saline. At day 42, the SAVE-5 and sham-vaccinated groups were challenged with PRRSV VR2385, and the experiment was terminated at day 54. Blood samples were collected on a regular basis for determining presence and amount of PRRSV RNA and PRRSV antibody levels. At necropsy tissues were collected and macroscopic and microscopic lesions were evaluated and compared across groups. The model to analyze continuous data collected over time was a repeated measures analysis of variance. A p-value of less than 0.05 was considered significant.
Results: SAVE5 was effectively attenuated as evidenced by a low magnitude of viremia and lack of nasal shedding of SAVE5 vaccine virus in any of the pigs. However, by day 42 only 40% of the vaccinated pigs had detectable anti-PRRSV IgG. After challenge, clinical signs, microscopic lesions, virus shedding and PRRSV viremia were not different between vaccinated and unvaccinated groups; however, vaccinated pigs (14.9±3.0) had significantly reduced macroscopic lung lesions compared to unvaccinated pigs (37.7±5.6).
Conclusion: Under the study conditions, the SAVE approach was successful in attenuating a PRRSV isolate and was also successful in reducing macroscopic lung lesions after homologous challenge compared to unvaccinated pigs. Although the results are encouraging, additional work needs to be done to further improve SAVE5 vaccine efficacy including testing other administration routes in growing pigs and testing the vaccine in pregnant sows.

Disclosure of Interest: None Declared

Keywords: Experimental Model, Porcine reproductive and respiratory syndrome virus (PRRSV), Vaccine

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-168

Divergence in the molecular diagnosis of Porcine Reproductive and Respiratory Syndrome virus

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Introduction: The Porcine Reproductive and Respiratory Syndrome virus (PRRSv) is a disease endemic on most porcine herds causing significant economic impact in the pig sector. Two genotypes were identified nearly simultaneously in Europe and the USA with antigenic differences that lead to two distinct genotypes: the European type (genotype 1) and the North American type (genotype 2).
Materials and Methods: The PRRSv infection status has been followed in Flemish voluntarily participating herds in 2015 (More details in companion abstracts 'Piglet Monitoring' in Northern Belgium). Forty two pools of 3 sera samples previously tested positive for PRRSv by means of the vetMAX PRRSV EU/NA real-time PCR assay (LSI) were retested individually (n=126) for PRRSv by 3 different RT-PCR tests including a conventional RT-PCR targeting the ORF5 region; a Sybr green RT-PCR assay targeting the ORF7 region and the commercial VIROTYPE real-time RT-PCR (Qiagen). The ORF5 amplicons were sequenced in order to confirm the present viral genotype. The Sybr green and Virotype PCR assays allow discrimination between PRRSv genotypes on basis of melting temperature (Tm) or probes specificity respectively.
Results: Among the samples only 44% were identified as positive by the 3 molecular assays. Considering the assays individually 84% of samples gave positive result with the conventional PCR, 68% with the Sybr green assay and 65% with the Virotype PCR assay.
Sequencing performed on partial ORF 5 amplicons (n=106) indicated the predominance of the genotype 1 (91%). The European type (n=96) was confirmed for 37% of the samples by the Sybr green assay and for 53% by the Virotype PCR assay whereas 13 and 12% of them were identified as genotype 2 by the same tests respectively and for 15 other % the Tm obtained in Sybr green assay did not allow a distinction between genotypes 1 and 2.
In parallel the Sybr green and the Virotype PCR assays have confirmed the North American genotype identification obtained by sequencing for 2 and 3 out of the 4 samples whereas one sample was identified as European strain by both tests.
Considering the results of pool testing by the fourth PCR assay (vetMAX, LSI) the accuracy of genotype identification was 83% for the conventional PCR assay; 58% for the Sybrgreen assay and 78% for the Virotype PCR assay.
Conclusion: The divergence observed between the different molecular assays is problematic as it demonstrated that none of the tested methods was efficient to ensure a confidant detection of virus presence in herds. Moreover it appears that both the melting temperature range from the Sybr green assay and the specificity of the probes could no more be considered as a confident criteria for genotype identification.

Disclosure of Interest: None Declared

Keywords: Diagnostics, PCR, Porcine reproductive and respiratory syndrome virus (PRRSV)

Viral and Viral Diseases
PRRS

PO-PW1-177

Integrative analysis of microRNA expression profiles in primary alveolar macrophages after infection with PRRSV strains of different virulence

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Introduction: Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), known as the causative agent of PRRS, is considered strain variation of different virulence. A class of small regulatory RNAs, termed microRNAs (miRNAs) was associated with gene regulation at the post-transcriptional level. It has been well established that miRNAs play many complex roles during viral infections. To indentify the impact of PRRSV strains of different virulence infections on the cellular miRNAome, we chose vJX143 and vJXM100 as research objects to perform deep sequencing and construct microRNA expression profiles from PRRSV-infected primary alveolar macrophages (PAMs). The present study has revealed the common and distinct PAM miRNA signatures associated with different virulent PRRSV infections and demonstrated that the relative expression level of miR-10b could be a potential biomarker for indicating different PRRSV strains.
Materials and Methods: vJX143 (at passage 3) was a highly pathogenic PRRSV strain isolated from the serum of a dying piglet in 2006. vJXM100 was a vJX143-derived attenuated PRRSV strain from MARC-145 passages. PAMs were infected with vJX143 and vJXM100 separately and total RNA containing the miRNA species were extracted from infected and mock-infected cells. Deep sequencing was performed by BGI Tech. The data were mapped to the known miRNAs of all organisms listed in the current miRBase and *Sus scrofa* genome. The relative expression levels of selected miRNAs were detected by quantitative real-time PCR.
Results: The results showed that a large and diverse group of miRNAs are expressed in three samples and that the expression of a subset of these miRNAs is altered in PRRSV infected macrophages. Twenty-eight annotated miRNAs had p values of < 0.05 and an absolute fold change ≥ 2, which indicated they were differentially expressed after vJX143 infection, while 58 miRNAs were differentially expressed after vJXM100 infection. Interestingly, miR-10b, which was proved an important regulator of many pathological processes, showed significant differentially expressed between vJX143 and vJXM100.
Conclusion: Our results provide a new insight into the differences of miRNAs response to different virulent PRRSV infection, and suggest a possible miRNA molecular signature associated with different PRRSV strains.

Disclosure of Interest: None Declared

Keywords: miRNA deep sequencing, miRNA molecular signature

Viral and Viral Diseases
PRRS

PO-PW1-169

DEVELOPMENT AND VALIDATION OF A PORCINE RESPIRATORY AND REPRODUCTIVE SYNDROME VIRUS CHALLENGE MODEL IN PIGS

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Introduction: Porcine Respiratory and Reproductive Syndrome Virus (PRRSV) is a disease which has a significant impact on the global pig industry. The objective of this work is to develop and validate an experimental challenge model for PRRSV to facilitate the efficacy testing of novel vaccines and therapeutics.
Materials and Methods: A total of 23, seven week (± 1 week) old piglets (PRRSV antibody negative) were included on the study. On arrival the animals were weighed, blood sampled and allocated to two groups of 10 and one of 3.
One week after arrival (on Day 0) the two groups of 10 were challenged by the intranasal route with 2ml of a PRRSV serotype 1, sub type 2 isolate (BOR or LT3) at a concentration of $1x10^6$ $TCID_{50}$/ml. Challenge was repeated on the next day.
Post challenge, clinical observations were carried out daily for 14 days.
On Day 14, the animals were euthanased and the lungs of each animal were removed.Gross pathological abnormalities were recorded. Lung samples were removed for virus isolation and histology.
Results: Post challenge rectal temperature increases were observed in 9 of the 10 animals from the LT3 group and 10 of the 10 from the BOR group on at least two occasions post challenge, with 8 animals from each group recorded to have high temperatures on at least two consecutive days. Only two observations of increased temperatures were recorded in total for the control animals during the monitoring period. Peak group mean temperatures were recorded on Day 6 post challenge. No other clinical abnormalities were observed during the monitoring period.
At necropsy all animals from the challenged groups were recorded to have swollen, congested lungs with pathology typical of PRRSV infection with mean total scores in the two groups of 12 and 12.7 respectively (out of a total of 20). The control animals had no visible pathology and scored as 0.
Conclusion: The study demonstrated that intranasal challenge with isolates of PRRSV serotype 1, sub-type 2 (BOR or LT3) is reproducible and consistent with a low level of variability between animals. The model will be applicable in the testing of veterinary medicinal products to control and prevent PRRSV infection in pigs.

Disclosure of Interest: D. Reddick Conflict with: Moredun Scientific, C. Ramage Conflict with: Moredun Scientific, S. Fraser Conflict with: Moredun Scientific, R. Macdonald Conflict with: Moredun Scientific

Keywords: Disease model, PRRS, Vaccine

Viral and Viral Diseases
PRRS

PO-PW1-141

Comparison of full dose versus partial dose of a modified live PRRS vaccine

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Introduction: High mortalities are sometimes observed in pigs exposed to PRRSv, even when vaccination is administered. Vaccination protocols at only half the label dose of modified live PRRS vaccine are used infrequently in the field to reduce production costs. Half dosing PRRS vaccine may lead to more variation in the protective immune response to field virus PRRS infections when faced with highly pathogenic strains and/or early exposure compared to a full dose of modified live PRRS vaccine. The objective of the project was to determine if variation in grower finisher mortality could be reduced in a flow experiencing high mortalities by utilizing a full dose protocol of a modified live PRRS vaccine compared to a half dose protocol.
Materials and Methods: Two flows experiencing higher than expected mortality in grower finisher pigs located in a hog dense portion of the upper Midwest were identified. Prior to June, all pigs received a half dose of a modified live PRRS vaccine 1-2 weeks post-weaning. Close out group data was collected from March through November for these barns. Starting in June all pigs in the same flows started receiving a full dose of a modified live PRRS vaccine 1-2 weeks post-weaning. Barn close out data on production performance was obtained. A total of 327 barns given the one half dose of a modified live PRRS vaccine and 136 barns given the full dose of a modified live PRRS vaccine were descriptively analyzed and statistical process control (SPC) charting conducted. Oral fluid (OF) samples were obtained from 100 barns vaccinated with a full dose at weaning, 7-8 weeks post-weaning, in the nursery, and 12-14 weeks post weaning in the finisher. Sequencing was done on PCR positive samples.
Results: OF results from 100 sites tested revealed 28% of the positive sites to be infected with field virus. Close outs on 327 groups that received one half dose of a modified live PRRS vaccine had a mortality rate of 5.69% from wean to finish compared to a mortality rate of 4.12% on 136 groups that received a full dose of a modified live PRRS vaccine.
Conclusion: Groups receiving the recommended full dose of a modified live PRRS vaccine had lower mortality and less variation than prior groups receiving a half dose of a modified live PRRS vaccine. If side by side comparisons are difficult to do, before and after Statistical Process Control (SPC) charting can provide a valuable tool in vaccine decisions when process changes are considered.

Disclosure of Interest: None Declared

Keywords: Dose, MLV, Vaccine

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-084

PRRS ARC & E in Small Farrow to Finish Operations in 3 connected counties in Alberta Canada.

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Introduction: This project is to first in Alberta and western Canada to eradicate PRRS from 3 adjoining counties with endemic PRRS infection problems from small continuous flow farms as well as multisite operations, and pursue the understanding of the economics of before and after PRRS positive status. We initially established that 13 of 25 sites were PRRS positive. We have now successfully eradicated PRRS in all of participating locations.

Materials and Methods: PRRS eradication in small family farms and 3 site systems was achieved through client adoption of the understanding of several fundamental PRRS eradication principles described below:
i) Stabilization of the Sow herd through herd closure minimum 8 months and internally derived stable gilts.
ii) Ingelvac PRRS MLV vaccination (BI) was strategically utilized for gilt stabilization in some herds and systems. Vaccination also contributed to the 'convincing' of producers that our PRRS strains, though considered 'PRRS-lite', were worth mitigating and or removing, despite previous opinions.
iii) Demonstrate 'PRRSV Sow Stability' through multiple serologic PCR evaluations of piglets at birth to weaning.
Eradication has been achieved with partial depopulation of nursery grower & finish pigs. Typically this procedure included the weaning to the offsite location for a certain period of time to facilitate the proper down time on the original F-F site for adequate clean up and proper disinfection procedures.
Through serological herd evaluation we established PRRS virus stability in the farrow to finish farm: The sero-profile typically has been:

- **30 individual suckling pig 1/litter**
- **10 Nursery (more samples if nurseries are all in all out)**
- **10 Grower (more samples if AIAO rooms)**
- **10 finisher (more samples if AIAO rooms)**
- **5 gilts prebreed, postbreed-mid pregnancy**
- **5 Sows of each parity out to 6+ parity**

Samples were all tested using the Idexx 3X PRRS EIISA test and pools of 3 for PRRS PCR testing. PCR positive samples had ORF 5 sequencing for reference.
Results: This project was inititated in 2004 with the first geographically safe location and has now been completed in all participating farms in the 3 counties as of Dec 2015. These producers now enjoy significant improvements in nursery-grow-finish performance, finish floor mortality, feed efficiency and financial barn close-outs. All sites have been involved in PADRAP assessments, producer education sessions on PRRS and ongoing biosecurity education.
Conclusion: Our ARC and E project involving 3 adjoining counties has served to demonstrate that PRRS eradication can be accomplished successfully in Western Canadian Swine herds.

Disclosure of Interest: None Declared

Keywords: Eradication, PRRS , Small Farms

Viral and Viral Diseases
PRRS

PO-PW1-136

PRRSV STABILIZATION OF A FARROW-TO-FINISH FARM EXCLUSIVELY THROUGH INTRADERMAL VACCINATION OF SOWS AND PIGLETS AND STRICT BIOSECURITY MEASURES

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Introduction: PRRSv stabilization of a herd through combination of mass-vaccination, batch to batch piglet vaccination and strict biosecurity measures has proven to be efficient. The following case report is the first to describe PRRSv stabilization through intradermal (ID) vaccination only combined with biosecurity implementation.
Materials and Methods: This field study was implemented in 2014 in a 300 sow farrow-to-finish farm located in a high swine density area. Piglets were weaned at 21 days of age every 2 weeks. Clinical signs resulting from an active EU PRRSv circulation were mild at the time of the diagnosis. In September 2014, the herd was closed for 8 weeks (no gilt entrance), strict biosecurity measures were implemented including unidirectional pig and human flow, and a specific PRRSv vaccination scheme using Porcilis® PRRS administered ID only was introduced. First, the whole herd (a total of 3340 sows and pigs from 21 days to end of fattening period) was mass-vaccinated twice 4 weeks apart. Thereafter, all the sows were mass vaccinated every 4 months, naive gilts were vaccinated twice: upon arrival and 4 weeks later, and 11 batches of piglets were vaccinated at weaning and 4 weeks later. The last batch of piglets was vaccinated in January 2015. Herd stabilization was confirmed by monitoring absence of PRRSv transmission from sows to piglets (RT-PCR on piglets at weaning) and between sows (using serological follow-up of sentinel gilts), starting March 2015, i.e 12 weeks after a previous mass vaccination of the breeding animals. In parallel, growers were serologically monitored for PRRSv antibodies.
Results: Monitoring results confirmed absence of PRRSv transmission from sows to their piglets. Sentinel gilts were accidentally raised in contact with recently vaccinated gilts, which interrupted the monitoring after 1.5 months. However, as no clinical signs were observed and PRRSv was not circulating from weaning to slaughter in 2 successive batches of growers, the farm was considered obviously stable.
Conclusion: As PRRS stabilization of this farrow-to-finish farm was achieved through exclusively vaccinating with Porcilis®PRRS by intradermal route, it can be concluded that this route is as efficient as intramuscular vaccination. Intradermal vaccination was also found very convenient by the farmer for its practicality and speed. Thereafter, the farm continued to intradermally vaccinate the sow herd every 4 months with Porcilis® PRRS.

Disclosure of Interest: None Declared

Keywords: PRRS; stabilization; intradermal

Viral and Viral Diseases
PRRS

PO-PW1-145

Field experiences with concurrent vaccination strategy aiming to control breeding performance in PRRS in large pig farms

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Introduction: PRRS virus was identified in 1990' earlier and classified two groups in type 1 and type 2 genetically. PRRS virus has been discovered all over the world. PRRS modified live virus vaccines are introduced in many country to control PRRS. But these PRRS live virus vaccines didn't defend against heterologous PRRS virus perfectly. This field case shows reproductive performance after type 1 modified live virus vaccination when it is infected with type 1 wild PRRS virus and its symptoms are severe while using type 2 modified live virus vaccine in a large farm.
Materials and Methods: This farms are separated in five reproductive farm independently (530, 980, 930, 930, 940 sows) in identical area. Each farm was already identified type 2 PRRS virus about 18% different from VR-2332 ORF5 sequence and have type 2 modified live vaccination to sows quarterly to control PRRS strategically(MLV, 2.0ml) . Type 1 wild PRRS virus was identified in Dec 2013. And in Jan 2014 type 1 modified live virus vaccine(Amervac, 2.0ml) was introduced. In 2014 type 1 and type 2 modified live virus vaccines(each vaccination 2.0ml) are concurrently used quarterly(in 2014 total PRRS modified live vaccination 10 times) and type 2 modified live vaccine were introduced in 3 weeks weaning piglets continuously. Serum samples were tested using PRRSV ELISA and RT-PCR was performed from blood samples before quarterly PRRS modified live virus vaccination Blood samples in sows 10, nursgery piglets 10, 40dys piglets 10 , 70dys piglets 10 and replacement gilts 10 are collected from each five farm.
Results: A year later after concurrent vaccination with MLV(2.0ml) and AMERVAC(2.0ml) no wild type PRRS virus was detected in the sera of the sows and replacement gilts but type 1 PRRSV or type 2 PRRSV are still detected in the sera of the nursery piglets, 40days pigs, 70days pigs But the performance of reproduction are significantly improved in 2014 compared with 2013 In 2013 total 616 sows in all five pig farms are aborted with infection of PRRSV In 2014 total 208 sows are aborted. And total mortality rate has reached 11.7% in 2013, but total mortality rate have decreased by 0.7% in 2014
Conclusion: Large-scale five farms are concentrated in identical areas and it is difficult to maintain biosecurity of these farms and prevent PRRSV infection outside. Despite of detecting PRRSV in piglets, 40days and 70days concurrent type 1 and type 2 PRRSV vaccination has enhanced breeding performance especially about 400 sows without abortion have contributed to increase production volume in farms.

Disclosure of Interest: None Declared

Keywords: None

Viral and Viral Diseases
PRRS

PO-PW1-109

Evaluation of the impact of PRRSv infection on growth performances of growing pigs in a panel of French herds

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Introduction: Holdtkamp (2013) estimated the cost of Type II PRRSv infection for the pig industry in the United States (US) to be 2,24€/marketed pig (based on 77% seroprevalence in US – ie ≈2,90€/marketed pig in positive herds, economic impact on sows excluded). In Europe, PRRSv type I is the most frequent and is generally considered as less virulent as type II. Few data exists on PRRSv type I economic impact. The objective of this study is to evaluate in a panel of French herds without specific clinical signs, the prevalence of PRRS infection and its potential economic impact on growing pigs.
Materials and Methods: This study was performed between September 2014 and June 2015 in Brittany (France). To be included in the study every farm has to have longitudinal registered data (wean-to-finish Average Daily Gain - ADG, Feed Conversion Ratio – FCR and Mortality).
In each herd, 10 pigs were blood sampled at the end of the fattening period (21 to 28 weeks of age) in order to establish PRRS status. Sera were pooled by 5 and assayed for PRRSv antibodies by Elisa (PRRS X3, Idexx). Given a prevalence of 30% of seropositive pigs in a population, a sample size of 10 pigs allows to detect the infection with a confidence level of 95%. A herd was defined "positive" if at least one pool was positive and "negative" if the two pools were negative.
Results: A total of 41 herds were included in the panel. Twenty two farms were classified as "negative" and 19 were classified as "positive". At the herd level PRRS prevalence is 46%. ADG was significantly higher in "negative" herds compared to "positive" herds (775 g/d versus 737 g/d, $p<0,01$), whereas differences in FCR and mortality were not statistically significant (2,48 versus 2,52 and 4,6% versus 4,9% respectively).
Conclusion: This study demonstrates the impact of PRRSv type I infection in growing pigs in France, with +38 g/d of ADG between "negative" and "positive" herds. Compared to Holdtkamp (2013) we show more impact on growth and less on mortality. Economically, with 2015 figures, differences in performances between "negative" and "positive" herds represent in this study +3,15€/pig.

Disclosure of Interest: None Declared

Keywords: Average Daily Gain, PRRSV

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-099

Particle size distribution of airborne PRRS and PED viruses emitted by infected animals inside and outside swine facilities

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) and porcine epidemic diarrhea (PED) viruses are airborne pathogens able to spread rapidly regionally. Despite this evidence, the information on the size of particles with which airborne viruses are associated while airborne in the field is limited. This association will determine the virus contribution to the airborne route, will help to explain the in between-farm transmission, as well as the survivability/infectivity of the viruses in aerosols. Thus, the objective of this study was to characterize the size distribution of the particles that transport PRRSV and PEDV generated by acutely infected pigs under field conditions.
Materials and Methods: Farms in Minnesota experiencing active outbreaks of PRRSV or PEDV were enrolled in the study during the winter of 2014-15. Two and 3 replicates of air samples/ room were collected for 1h using an 8 staged Andersen cascade impactor (ACI, 28.3 lpm) and for 15min using a 5 staged high volume Tisch cascade impactor (TCI, 1,100 lpm), respectively. Both air samplers,are capable to collect inhalable particles as function of particle size (from 0.01-10µ). Samples were collected inside and at 5m outside the farms. Additionally, an optical particle counter was used to analyze total airborne particles during the sampling periods. Samples were analyzed by quantitative PRRSV and PEDV RT-PCR. Log of total quantity of RNA virus/m^3 of air, and total particle counts by size and type of virus were analyzed for the study.
Results: A total of 210 air samples from 4 nurseries were analyzed by quantitative RT-PCR. PEDV farms had no clinical signs at the time of sampling; however it was detected in the air in all particle sizes measured inside and outside and in higher quantities than PRRSV. Inside, PEDV was detected in quantities ranging from 2.8 logs (particles between 0.7-1.1µm) to 6.25 logs (4.7-5.8µm). PRRSV, was detected in all size ranges, except for particles < 2.1µm (limit of qPCR detection was added for calculations) in quantities ranging from 2.4 (2.1-3.3µm particles) to 5.4 logs (particles >9.0µm). All samples collected outside were negative for PRRSV.
Conclusion: Results from this study indicate that PEDV and PRRSV can be found in a wide range of particle sizes while airborne under field conditions, however, PRRSV seems to be essentially associated to larger particles. PRRSV airborne particles were not detected outside the swine facilities. This information is important to further our understanding of airborne transmission and to evaluate the efficacy and impact of biosecurity measures such as air filtration on the risk of pathogen introduction in filtered farms.

Disclosure of Interest: None Declared

Keywords: Air samplers, Particle size, Viral aerosols

Viral and Viral Diseases
PRRS

PO-PW1-105

Characterization of PRRS viremia and shedding in naïve replacement gilts inoculated with PRRS RFLP 1-7-4

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Introduction: A porcine reproductive and respiratory syndrome (PRRS) restriction fragment length polymorphism pattern of 1-7-4 emerged in North Carolina in 2014. The highly virulent nature of the virus was evidenced by a wide-ranging loss of production in breeding herds regardless of prior PRRS immune status. Mitigation of risks associated with introduction of PRRS naïve replacement gilts into a breeding herd has been attempted with vaccination or intentional exposure of wild type isolates. The purpose of this study was to characterize the viremia and shedding in naïve replacement gilts given PRRS RFLP 1-7-4 live virus inoculation (LVI) only and those given commercial modified-live PRRS vaccine (MLV) four weeks prior to LVI.
Materials and Methods: Two gilt development units (GDUs) located off-site from the two destination sow farms were selected and assigned treatment groups. Each GDU held 450 naïve gilts ranging from 12-22 weeks of age. GDU 1 received LVI only upon arrival and GDU 2 received MLV upon arrival and LVI four weeks later. The PRRS 1-7-4 virus used for inoculation was collected from acutely infected wean pigs from the corresponding GDU's destination sow farm. The LVI products were formulated to an infectious dose of 10^3 virus particles/mL and each gilt was given 1 mL IM. The gilts receiving MLV were given 2 mL IM of a commercial modified-live PRRS vaccine. At each GDU, blood (viremia and seroconversion) was collected from 45 tagged gilts and 10-12 oral fluids (shedding) were collected weekly to bi-weekly until all sample types were PCR negative. Serum and oral fluids were tested by PRRS qPCR and ELISA. PRRS sequence analysis was done one week post-inoculations to confirm development of viremia to MLV or LVI respectively.
Results: PRRS PCR and sequencing for all sample types at one week and ELISA at three weeks post-inoculation were all positive confirming infection and seroconversion to the inoculum given at arrival. PCR cycle time (Ct) values of serum and oral fluid samples one week post-LVI were 23.6 and 26.7 for GDU 1 and 30.4 and 31.2 for GDU 2. All samples types were negative at 12 weeks post-LVI for GDUs 1 and 2. Total mortality was 29 (6.4%) and 7 (1.5%) for GDU 1 and GDU 2 respectively.
Conclusion: PRRS 1-7-4 LVI time to negative viremia and shedding was 12 weeks under the conditions of this study, regardless of prior immune status. Use of MLV four weeks prior to LVI may reduce viremia and shedding levels as suggested by higher Ct values one week post-LVI. Use of MLV four weeks prior to LVI may reduce mortality associated with PRRS 1-7-4 infection.

Disclosure of Interest: None Declared

Keywords: Intentional Exposure, PRRS, viremia, shedding, Replacement Gilts

Viral and Viral Diseases
PRRS

PO-PW1-155

Impact of PRRS RFLP 1-7-4 in previously positive and vaccinated versus naive sow herds

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Introduction: A porcine reproductive and respiratory syndrome (PRRS) restriction fragment length polymorphism pattern of 1-7-4 emerged in North Carolina in 2014. The purpose of this study was to evaluate the impact of PRRS 1-7-4 in previously positive sow herds versus naïve sow herds using sow performance data.
Materials and Methods: Sixteen sow farms diagnosed with PRRS 1-7-4 over a 10 week period during spring 2015 were selected. Prior to PRRS 1-7-4 break, 6 farms were Status II-A (Positive) and were routinely vaccinating with a commercial modified-live vaccine (MLV). Ten farms were Status IV (Negative) prior to the break. The post-break intervention strategy included MLV vaccination and was the same for all farms.
Sow production data was collected from 13 weeks pre-break to 24 weeks post-break for each farm. The data was compared between the Positive and Negative groups during the active PRRS break period which was either 10 or 20 weeks depending on behavior. The 10 week active period parameters were abortions, sow mortality, stillborns per litter farrowed and pre-wean mortality. The 20 week active period parameters were pigs born alive per litter farrowed, mummies per litter farrowed, pigs weaned per litter farrowed and total pigs sold. Time to baseline production (TTBP) and time to stability (TTS) were also measured.
The data was analyzed using ANCOVA where baseline production (average of 13 weeks prior to break) was used as a covariate to evaluate the differences in groups for each parameter. For both groups, the parameter data was reported as least square means for the active period defined for each parameter. The level of significance was set at 10% for all tests.
Results: A significant increase in abortions was observed in the Positive group (+5%, p=0.05), however, there were significantly fewer losses observed in pigs born alive per litter farrowed (+1.41, p=0.01), pigs weaned per litter farrowed (+2, p<0.01) and total pigs sold (+185.60, p=0.02). The Negative group had a significant increase in stillborns per litter farrowed (+0.61, p=0.01), mummies per litter farrowed (+1.17, p=0.04) and pre-wean mortality (+15%, p=0.03). There was no significant difference in sow mortality between the two groups. Average TTBP was 15.4 and 22.9 weeks for Positive (n=5) and Negative (n=10) groups, respectively. Average TTS was 25 and 24.5 weeks for the Positive (n=4) and Negative (n=6) groups, respectively.
Conclusion: The sow performance data of the Positive group was significantly better than those in the Negative group for all production parameters except abortions and sow mortality under the conditions of this study. TTBP was shorter in the Positive group and TTS was similar between groups at 24 weeks post-break.

Disclosure of Interest: None Declared

Keywords: Prior Immunity, PRRS RFLP 1-7-4, Sow Performance

Viral and Viral Diseases
PRRS

PO-PW1-119

Explanation of apparent PRRS virus recurrence during the 174 U.S. epidemic – an introduction to MJPRRS® Grouping Technology

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Introduction: Since late 2013, a RFLP 174 PRRS virus epidemic (epi) has spread across the U.S. This epi has shown highly conserved virus RFLP (cvRFLP) and been clinically aggressive. Analysis suggests that farms have been re-infected over a short period of time with the same virus (RFLP, nucleotide [Nt] homology, and dendrogram [Dg] comparisons). These methods have failed to explain the clinical changes occurring within the swine populations (pop(s)).
Materials and Methods: *MJPRRS®* Grouping Technology (MJGT) is a unique means of comparing and classifying PRRSv based on ORF 5 amino acid (AA) sequences and on-farm clinical signs (cs). Developed by Dr. Kim at MJ Biologics and licensed to Phibro in Jan 2015, this method of "grouping" PRRS viruses has helped producers better understand key biosecurity intervention points, control pig flow health, and maximize system throughput.
Application of *MJGT* to this epi helps explain how similar viruses demonstrate varied cs within a PRRSv positive swine pop. *MJGT* is a tool for classifying and differentiating PRRSv based on their different physical properties identified in the ORF 5 AA sequence. All PRRSv that have been analyzed fit neatly into 1 of known 25 MJ groups. *MJGT* has identified common mutations and has facilitated tracking of epis even where Nt comparisons and RFLP analysis would suggest that different PRRSv may be involved.
Results: From Dec 2014 through Oct 2015, 91 PRRSv sequences with an RFLP of 174 were analyzed using *MJGT*. Analysis determined that 86% of sequences had an RFLP of 174 and a D7 *MJGT* group. The remaining sequences were identified as belonging to D2, D4, or D6 groups. Group changes would be expected to demonstrate cs in a portion of the pop even with cvRFLP. Farms experiencing a D7 of 174 RFLP, and then a recurrence of the 174 RFLP, have exhibited such a group change.
Evaluation was done on PRRSv sequences that had been identified as a D7-grouped virus. A total of one hundred twenty eight sequences were identified as D7 over the same period of time, regardless of RFLP. Of these D7 viruses, 61% were both D7 and RFLP of 174. Where a 174 RFLP and D7 group were not aligned, RFLPs varied: 144, 184, 173, 172, and 164.
Conclusion: This technology supports observations that not all 174 PRRSv are created equal. Despite cvRFLP across the U.S., variation in viral presentation was observed. *MJGT* helps explain the recurrence of cs without a change in RFLP.
MJGT compares physical viral protein presentations. Changing MJ PRRS groups often correspond to clinical signs in a pop. Using *MJGT* has the capability to explain why clinical changes occur in a pop where Nt homology, RFLP, and Dg proximity fail to demonstrate differences in the genetic make-up of the virus.

Disclosure of Interest: K. Kinsley Conflict with: Phibro Animal Health Corp, D. Guggenbiller Conflict with: Phibro Animal Health Corp, D. Weiss Conflict with: Phibro Animal Health Corp, R. Nimmo Conflict with: Phibro Animal Health Corp, B. K. Kim Conflict with: MJ Biologics

Keywords: 1-7-4, PRRS, Vaccine

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-127

Influence of Ingelvac® PRRS MLV vaccination on variability of S/P values serology in a breeding herd

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Introduction: PRRS modified live vaccine plays an important role in controlling Porcine Reproductive and Respiratory disease. Serology is a useful monitoring tool to assess PRRS exposure dynamic herds under a PRRS stabilization program. This study describes SP values variation during 5 years in a farrow to finish farm applying Ingelvac® PRRS MLV as a primary tool for achieving stabilization.
Materials and Methods: The study was conducted in a 13-year old farm with 1500 sows single-site with continuous flow system located in east China. In 2006, this farm suffered an outbreak with highly-pathogenic porcine reproductive and respiratory syndrome (HP-PRRS) and the main symptoms were abortions and high mortality in lactating and nursery pigs. In February 2009, Ingelvac® PRRS MLV was implemented where breeding herd was mass vaccinated every 3 months while the piglets were vaccinated at 14 days of age. 100 blood samples annually distributed in 20 gilts, 20 sows from Parity 1-2, P 3-4, P 5 and P 6+ respectively were taken and tested for IDEXX PRRS. The S/P values, weaned pigs per sow and growing mortality were analyzed at the beginning of MLV implementation in 2009 and during the control program (2010, 2011, 2012, 2013 and 2014) using MINITAB 16.2.3 (State College PA USA), SP values were analyzed in BoxPlot chart and Kruskal-Wallis analysis.
Results: A clear reduction of variability of SP values at each sampling point was observed. S/P values median was statistically significant reduced along the line. The variation of S/P values was also reduced (Inter Quartile Range from 1.141 to 0.600) through the vaccination period. This reduction of variability on S/P values matches with productivity improvement where there were 3.1 more pigs weaned per sow and the growing mortality was reduced from 4.3% to 1.5%
Conclusion: Breeding herd stability can be defined as a reduction of PRRS resident virus circulation within the population. This is an important milestone in any PRRS control program. Considering that IDEXX PRRS ELISA measures exposure, the reduction of variability in S/P ratios along the line, can be interpreted as a reduction of circulation-exposure of resident virus that reflects a stronger stabilization process in the breeding herd (no resident virus circulation). This farm has been using Ingelvac® PRRS MLV since 2009. This is an innovative and practical way to analyze serology as stabilization measurement tool in vaccinated breeding herds1. In addition, during the period of 2011-2012, the farm suffered less losses compared to other farms co-infected with PED and Aujeszky's disease.

Disclosure of Interest: None Declared

Keywords: Vaccination, vallues, variability

Viral and Viral Diseases
PRRS

PO-PW1-092

A WUR SNP is associated with European Porcine Reproductive and Respiratory Virus Syndrome resistance and growth performance in pigs

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) is the most economically significant disease impacting pig production worldwide. Methods other than vaccination must be also explored to aid the control of this disease; one possibility is genetic improvement of the host to decrease the negative impact of this disease. More recently, the heritability for viral load was estimated to be close to 0.3 after challenge with a North American PRRSV strain. This trait and the average daily gain (ADG) were associated with a single genomic region in chromosome 4 (SSC4) which is best represented by the SNP tag marker WUR10000125 (WUR), located in the 3' non-coding region of the interferon-inducible guanylate-binding protein 1 (*GBP1*) gene. Presently, the effect that this marker could have on the ADG in PRRSV-free pigs is unknown. Moreover, there is scarce of information about the genetic resistance to European PRRSV strains. Threfefore, the main objective of the current study was to investigate the association of the WUR SNP at the *GBP1* gene with ADG in PRRSV infected and uninfected pigs.
Materials and Methods: The experimental procedure consisted of two trials. In the first one, after a 7-day acclimation period (day 0), pigs (n=40) were vaccinated intramuscularly with a commercial PRRS modified live vaccine (Porcilis® PRRS, MSD Animal Health). Blood samples were collected at 0, 4, 7, 10, 14, 21, 28, 35, and 42 days post-vaccination (DPV). Body weight was collected at 0 and 42 DPV. PRRSV viremia was measured using a semi-quantitative TaqMan PCR assay for PRRSV RNA. Viral load and ADG were determined for each pig. In the second trial, the ADG for PRRSV-free pigs was monitored during the rearing period. All pigs were genotyped for WUR at the *GBP1* gene (AA and AG genotype were defined). The effect of the SNP WUR on VL and ADG was estimated using a linear model including, the WUR genotype (AA, AG) and the weight at beginning of the trial and then tested following an F-test.
Results: After administering an attenuated PRRSV strain, PRRSV was detected in serum, at least once, in 22 out of 40 piglets (55%). Unfortunately, it was not feasible to determine VL as only 10% of the pigs had enough positive sample-points to calculate this trait. WUR genotype was associated to ADG during the first 42 DPV, with AG piglets performing better than the AA ones. On the contrary, The AA pigs showed higher ADG than the AG pigs in PRRSV negative environment.
Conclusion: There is a scope for selecting pigs according to their responses to PRRS virus infection with European strains and that WUR SNP may play a role in causing PRRSV resistance.

Disclosure of Interest: None Declared

Keywords: Genetic Resistance, PRRSV, SNP

Viral and Viral Diseases
PRRS

PO-PW1-088

Feasibility and performances of oral fluid and blood samples associated with commercial ELISA for PRRS antibodies detection in group housed sows

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Introduction: PRRSV leads to huge economic losses for the swine industry worldwide. Availability of simple, easy-to-use and accurate collection methods and laboratory tests are crucial for efficient PRRS diagnosis and monitoring. Blood sampling is currently the most frequently used method in the field for these purposes. A welfare friendly collection method, namely oral fluid (OF), has recently gained interest in the field as an alternative technique to blood sample. However, the feasibility and the diagnostic performances of this technique for PRRS antibodies detection have not yet been assessed for group housed sows and compared to those of blood sampling. Thus, the aim of this study was to assessand compare the feasibility and the diagnostic performances of 1/OF collection at the animal level associated with a commercial ELISA and 2/blood samples coupled with a serum-ELISA to detect PRRSV antibodies in group housed sows.
Materials and Methods: The study was carried out in 35 French breeding herds infected (32 herds) or non-infected (3 herds) by PRRSV. In every herd at least 30 gestating group housed sows and 3 pens were sampled at random. OF and blood were collected from each sow. OF and blood sampling times were recorded. OF and serum samples were analysed by commercial ELISA specific to each specimen (PRRS OF and PRRS X3, IDEXX, Eragny sur Oise, France). A Bayesian approach was used to estimate the sensitivity and specificity of each ELISA method without gold standard.
Results: A total of 1598 sows were sampled. On average, individual OF sample took 2 minutes 57 seconds (sd=2min 55s) per sow (one investigator) while blood sample took 1min 16s (sd=1min 03s) (two investigators required). The sows chewed the OF sampling device during on average 1min 36s (sd=1min 06s). Aggressive behaviours of group housed sows towards the investigators were observed during blood sampling in 37% of the herds whereas OF collection was peaceful in every herds. Although ELISA used on individual OF showed on average the same level of sensitivity (Se) than serum-ELISA (mean Se=95%), it lacked specificity (Sp) when compared to serum-ELISA (mean OF-ELISA Sp=84% and mean serum-ELISA Sp=94%).
Conclusion: OF sampling appears to be a more welfare friendly technique than blood sampling in group housed sows and is a promising tool for increasing the efficiency and cost effectiveness of PRRS infection surveillance in swine herds once the performances of the lab tests will be improved.

Disclosure of Interest: None Declared

Keywords: PRRS, diagnostic, oral fluid, serum, ELISA

Viral and Viral Diseases
PRRS

PO-PW1-089

SIMULTANEOUS VACCINATION WITH PRRS MLV AGAINST BOTH PRRSV TYPE 1 AND TYPE 2: PRRSV IN LUNGS FOLLOWING CHALLENGE

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Introduction: Both type 1 (subtype 1) and type 2 PRRSV are currently circulating in Denmark. In some double-infected herds, the pigs are simultaneously vaccinated with PRRS modified live vaccines (MLV) against type 1 and type 2. The objective of this experimental study was to compare the efficacy of the two vaccination strategies following challenge with homologous and heterologous virus strains.
Materials and Methods: Sixty-six, four-week-old PRRSV-negative pigs were included in the study. The pigs were purchased from a specific pathogen-free herd and verified free of a range of pathogens including PRRSV by serology. The pigs were housed at experimental animal facilities under appropriate biosecurity conditions. On arrival (week 0), the pigs were randomly distributed into four groups. Each group was housed in a separate room. One week after arrival (week 1), the pigs in groups 1-3 were vaccinated against PRRS type 1, PRRS type 2 or both. The last group was kept as a non-vaccinated control group. Nine weeks after vaccination, all pigs were divided into three new groups. The pigs were then challenged with either PRRSV type 1 subtype 1, PRRSV type 1 subtype 2 or PRRSV type 2 viruses. Two weeks later, all pigs were euthanized and macroscopic examinations completed. Samples were collected from three different sites in the lungs and tested for PRRSV load by real-time RT-PCR. Virus from at least one lung sample from each challenge group was sequenced.
Results: Only three pigs had interstitial pneumonia at autopsy, and they all belonged to the non-vaccinated group challenged with PRRSV type 2. Overall, there were no significant differences in the prevalence of real-time RT-PCR positive lung samples in each of the challenge groups when comparing the vaccination backgrounds. The results of the viral sequencing are ongoing, but the results obtained so far have revealed that the virus detected in the lungs was identical to the challenge strain and different from the vaccine strain.
Conclusion: None of the PRRSV vaccines were able to significantly reduce the prevalence of lungs positive for PRRSV RNA two weeks after PRRSV challenge in contrast to the effect of vaccination on virus shedding.
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Disclosure of Interest: None Declared

Keywords: Challenge, Lungs, Vaccination

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-087

Oral fluid samples for the monitoring of PRRSV status and dynamics

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Introduction: Approximately 40 % of Danish swine herds are positive for PRRSV. PRRSV is secreted in nasal and oral fluid and can be detected in serum for weeks after infection. To control PRRSV it is critical to monitor the PRRSV status in different production stages. Information on ongoing PRRSV infection is normally obtained by test of serum which is laborious and stressful for the pigs. The aim of the study was to test if test of oral fluids can replace serum for PRRSV monitoring in Danish sow herds.
Materials and Methods: A total of 14 PRRSV positive sow herds accommodating a nursery section were included. In each herd, oral fluids (1 per pen) and blood samples (pooled from 5 pigs per pen) were collected from four pens in each of three age groups of weaner pigs. The samples were subsequently tested for PRRSV by a specific real time RT-PCR assay. The pen level agreement between the test result in the two types of sampling material (serum and oral fluid) was analysed with the Kappa statistics.
From the herds where at least one serum sample tested positive for PRRSV, one isolate of PRRS was sequenced for ORF5. The sequences were analyzed phylogenetically.
Results: Of the 14 herds, 9 tested positive for PRRSV in both oral fluid and in serum in at least one of the pens. Similarly, in two herds, all oral fluid samples and all serum samples were negative in all pens tested. In one herd, at least one pen tested positive in oral fluids despite being negative in all serum samples and similarly, in one herd at least one pen tested positive serum in despite all oral fluids tested negative.
In total, paired samples of serum and oral fluid were available from 147 pens. Sixtyfour (64) pens were positive in serum and of those 61 were positive in oral fluid, too. Sixty-nine (69) pens tested positive in oral fluids. Seventy-five (75) pens tested negative in both serum and oral fluid. The Kappa value for agreement between the two types of sampling material was 0.85, which translates to “Almost perfect” agreement.
ORF5 sequencing of four Type 1 isolates showed that they all clustered together with other Lelystad-like Type 1 viruses from Denmark. Sequencing of the Type 2 viruses is ongoing.
Conclusion: The results showed that oral fluid is as sensitive as serum samples for the monitoring of PRRSV status and dynamics in Danish sow herds.

Disclosure of Interest: None Declared

Keywords: Diagnostics, Oral fluids, PRRSV

Viral and Viral Diseases
PRRS

PO-PW1-106

Comparison of three different air sampling systems for the detection of aerosolized type 1 PRRSV MLV

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Introduction: Airborne transmission of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) has been known for a long time. Most experiments were performed using type 2 PRRSV and fairly little information is available on the spread of type 1 PRRSV via aerosols. The aim of this study was to compare 3 different air sampling systems for their ability to detect aerosolized type 1 PRRS modified live vaccine virus.
Materials and Methods: Three different air sampling systems were tested: Coriolis®µ (flow rate: 3 m^3/10 min), Sartorius MD8 Airscan Air Sampler (flow rate: 1 m^3/10 min), and IOM Multidust sampler with polycarbonate filters (flow rate: 2.5 l/min; pore size: 0.2 µm). In an experimental chamber (air volume 68.5 m^3), one liter of PBS containing 1 x 10^7 $TCID_{50}$ (2.6 x 10^{11} virus copies measured by qRT-PCR) of a type 1 PRRSV MLV (ReproCyc® PRRS EU) was nebulized for 10 minutes with a cold-fogging system (UNIPRO² Igeba). Air sampling with 2 IOM Multidust samplers started simultaneously with nebulisation and continued for a period of 2 hours. Two air samplings of 10 minutes each were performed with Coriolis®µ and Sartorius MD8, the first during nebulisation and the second 1 hour later. Cell culture medium (MEM + 3 % FCS) was used for Coriolis®µ (15 ml) and to dissolve gelatine filters used in the Sartorius MD8 (20 ml) or to wash polycarbonate filters (10 ml). The medium was tested for the presence of PRRSV by qRT-PCR and virus isolation on MARC-145 cells.
Results: Relative humidity in the experimental chamber was 34 % at an air temperature of 22.8°C. The highest concentration of virus particles was measured by qRT-PCR in samples collected with Coriolis®µ (9 x 10^8 virus copies/m^3 air during nebulisation, 2.4 x 10^8 one hour later). In Sartorius MD8 samples 3.0 x 10^8 and 1.1 x 10^8 virus copies/m^3 air were measured during and after nebulisation. In samples of the 2 IOM Multidust samplers virus concentrations were 2.1 x 10^7 and 1.7 x 10^7 copies/m^3 air. PRRSV could successfully be isolated from the sample collected during nebulisation with Coriolis®µ (1.5 x 10 $TCID_{50}$/ml).
Conclusion: All three systems were able to detect type 1 PRRSV by qRT-PCR under experimental conditions. Virus isolation was only possible from a sample collected with Coriolis®µ. The same air sampling systems were tested in a PRRSV positive farm but failed to detect field virus in nursery and finishing rooms in which pigs were tested positive for PRRSV in oral fluids and blood. Further studies are necessary to investigate air sample collection in the field and to optimize sample collection and processing in order to evaluate the role of aerosols in transmission of type 1 PRRSV isolates.

Disclosure of Interest: None Declared

Keywords: PRRSV, airborne, air sampler

Viral and Viral Diseases
PRRS

PO-PW1-116

Humoral and cellular immune responses in gilts after intradermal application of Porcilis® PRRS at two different application sites

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) is one of the most challenging diseases in pigs. Vaccination against PRRS has been established as an important tool to minimize reproductive failure in breeding animals and respiratory diseases in growing pigs. The aim of this study was to evaluate humoral and cellular immune responses of gilts after intradermal application of live attenuated Type 1 PRRSV vaccine at two different application sites under field conditions.
Materials and Methods: PRRSV negative gilts were vaccinated intradermally with Porcilis® PRRS (0.2 ml) using a needle free device (IDAL) either in the neck (n=10) or perineal region (n=10). Blood samples were collected 28 days post vaccination to assess both humoral and cellular immune responses. Neutralizing antibody titers (nAbs) were measured by the use of fluorochrome-conjugated monoclonal antibodies against PRRSV nucleoprotein to test rest-infectivity of vaccine virus after incubation with serially diluted test serum on MA104 cells. The number of PRRSV-specific IFN-γ secreting cells was analyzed by ELISpot assays.
Results: Neutralizing antibodies were detected in 8/10 pigs vaccinated in the neck region with titers ranging from 1:22 to 1:256. Only 2 gilts reached high titers of 1:256 while the remaining 6 gilts were below 1:100. Due to bacterial overgrowth during incubation, nAbs could not be evaluated in 2 gilts vaccinated in the perineal region. The remaining 8 gilts were all positive for nAbs. With the exception of one gilt, that had a titer of 1:16, all gilts had high nAb titers which ranged from 1:112 to 1:512. PRRSV-specific IFN-γ producing cells were evident in both groups. The frequency of PRRSV-specific IFN-γ producing cells was somewhat higher in pigs vaccinated in the neck (25 to 96 spots within 3 x 10^5 peripheral blood mononuclear cells following subtraction of spontaneous IFN-γ producing cells in control cultures) compared to gilts vaccinated in the perineal region (4 to 90 spots). However, these differences were not statistically significant.
Conclusion: The intradermal administration of Porcilis® PRRS induced humoral and cellular immune responses independent of administration site. Therefore, the perineal region can be an alternative application site for intradermal application of Porcilis® PRRS to gilts.

Disclosure of Interest: None Declared

Keywords: PRRSV vaccination, intradermal application, immune response

Viral and Viral Diseases
PRRS

PO-PW1-135

Vaccination with Ingelvac PRRSFLEX® EU in four week old piglets showed efficacy during a naturally occurring field infection in Spain

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Introduction: Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is one of the major pathogens in pigs that have a significant economic impact on the swine industry worldwide. Vaccination against PRRSV has been demonstrated as an effective tool to control clinical signs related to infection. The aim of the present study was to evaluate field efficacy of a new PRRS genotype 1 modified live virus vaccine (Ingelvac PRRSFLEX® EU) in piglets at four weeks of age.
Materials and Methods: The study was conducted in a fattening farm in Spain with a history of PRRS infection. A total of 1364 piglets were included in the study and assigned to two groups (vaccinated and non-vaccinated control animals). Piglets were followed for average daily weight gain as well as clinical signs, mortality and concomitant treatments.
Results: Field infection with PRRSV occurred either before vaccination or shortly after. Peak viremia occurred around four weeks post vaccination. Vaccinated piglets showed a significant increase in average daily weight gain (ADWG) during peak of field infection (495 vs. 486 g/d). In addition, the group of vaccinated piglets showed a significantly reduced mortality rate (4.9 vs 6.1%) and frequency of concomitant treatments (18.6 vs. 23.0%), respectively. Furthermore, the proportion of pigs showing any abnormal clinical sign at least once at any of the examination time points was significantly lower in vaccinated pigs than in control pigs (4.2 vs. 8.3%), with special emphasis on respiratory signs that were significantly reduced in vaccinated piglets as well (2.3 vs 4.7%).
Conclusion: This study established that protective immunity was induced in vaccinated pigs as early as 4 weeks after vaccination in the face of an ongoing field infection. While a clear beneficial impact on clinical and productive parameters was measured in the face of PRRS viremia, the importance of stabilizing the breeding herd to reduce the vertical transmission of PRRS to piglets is critical to the long term disease control.

Disclosure of Interest: None Declared

Keywords: Ingelvac PRRSFLEX EU , PRRS type1 vaccine

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-151

Growing performance improvement after using PRRS MLV at 3weeks old piglets in a Korean swine farm

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) causes respiratory disease in nursery and grow-finisher pigs and reproductive failure in sows and boars[1]. PRRSV-infected pigs usually suffer from poor growth performance and are highly susceptible to co- or secondary bacterial and other viral infections[2]. PRRSV was first isolated in Korea in 1994 and all PRRSV isolates corresponded to the Type II until 2000, TypeIPRRSV has recently emerged in Korea[3]. In this study we evaluate the efficacy of Ingelvac® PRRS MLV (TypeII PRRSV based vaccine) piglet vaccination in a Korean swine farm infected with Type IPRRSV.
Materials and Methods: This study was conducted in a commercial 750 sow farm with multi-site rearing systems which has many different contracted finisher farms. End of 2012, the farm experienced a negative impact in productivity due to infection with TypeIPRRSV. The farm were already using FLEXcombo®(Ingelvac CircoFLEX® and Ingelvac MycoFLEX®) in 3 weeks old piglets, therefor it was obvious to use 3FLEX® (Ingelvac CircoFLEX® and Ingelvac MycoFLEX® and Ingelvac® PRRS MLV) at weaning. From Sep 2013 onwards, vaccination was performed with 3FLEX®(2ml) at 3 weeks old piglets. This farm already implemented quarterly mass vaccination in the breeding herd with Ingelvac® PRRS MLV. Before/After vaccination, blood samples were taken for antibody examination by ELISA(IDEXX PRRS 3X) and virus detection by RT-PCR. Performance data was analyzed before and after PRRS MLV piglets vaccination period
Results: Until Ingelvac PRRS MLV piglet vaccination was implemented, only TypeIPRRSV was detected in nursery houses. Before vaccination, seroconversion was taking place ealy in growing pigs. After piglets vaccination, PRRSV were detected only late period of nursery and the variability of SP vales was reduced, suggesting a better stabilization process. Performance was an improved after vaccination. Unfortunately, there was fired at nursery in Oct 2014. Although include that accident, the average growth rate was 1.98%, 7.51% higher in piglets vaccinated group(89.66% vs 91.64% vs 97.17%). Also, after PRRS MLV piglet vaccination at 21 days, we could reduce mortality at grow-finisher farms.
Conclusion: After the implementation of Ingelvac® PRRS MLV piglets vaccination, PRRS stabilization was achieved and an overall improvement in productivity was observed. This field case provides strong evidence of the ability Ingelvac® PRRS MLV to control TypeIPRRSV a Korean farm . Implementation of a 3FLEX vaccination program can be a beneficial method to solve PRRS problems in the nursery. And on top reduce vaccination labor costs.

Disclosure of Interest: None Declared

Keywords: 3FLEX, piglet vaccination, PRRS

Viral and Viral Diseases
PRRS

PO-PW1-120

Vaccination with PRRS type 2 strain against high pathogenic type 1 virus in growing pigs in Korea

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Introduction: In South Korea, type 2 PRRSV has evolved continuously and has been subsequently characterized into at least four lineages, ever since the infection by this PRRSV genotype was first described in 1993[1].
Recently, it was revealed that type 1 PRRSV to be not only widely distributed in South Korea but also divided into three genetic clusters based on phylogenetic analysis [2]. Martínez-Lobo et al.[3] emonstrated that pigs infected with the type 2 PRRSV strain showed more severe respiratory clinical signs and macro- and microscopic lung lesions than pigs inoculated with the type 1 PRRSV strain. In Korea, since initially reported of type 1 PRRSV and some pathogenicity but highly pathogenic type 1 strain has not been reported in South Korea[4][5]. Recently, relatively high mortality case with type 1 virus was identified [6]. This case report is the consequences of the vaccination of PRRS type 2 MLV against this highly pathogenic PRRS type 1.
Materials and Methods: The case farm was a two-site production system(i.e., a farrow-to-grower unit with 400 sows and a grower-to finish unit: moving 80 days old to growing unit: most of Korean 2-site farm is similar moving age) in central region of South Korea. The grower-to-finish unit was located 10 km east of the sow herd. The sow farm was historically free of PRRSV and PRRSV vaccine had not been used in both units. In October 2014, the grower-tofinish unit experienced an outbreak of acute respiratory disease in the growing pigs. The onset of the disease was observed at about 3 weeks after the arrival of the pigs with severe respiratory signs including high fever(41–42°C), dyspnea, coughing, and emaciation. When the mortality rate reached 22%, florfenicol and marbofloxacin administered. Although mortality rate was a little bit decreased, clinical signs were still observed in most pigs. Laboratory test confirmed that the single infection of PRRSV type 1 and there was no other respiratory causative agent(PCV2, CSF, App, Pm etc.).
The first batch of pigs were vaccinated with Ingelvac® PRRS MLV in November 4th 2014, 3 weeks after
placement in the grow finisher The following 6 batches, which had 2 weeks interval, were vaccinated on the date of arrival.
Results: The average mortality rate in 2014 was 12%. It had declined by intensive medication, but mortality rate was rising again since the second half of 2014.
After vaccination the mortality rate and clinical symptoms dramatically improved. The average mortality of the vaccination group was 2.02%.
Conclusion: In this study, it is concluded that a Type 2 strain based modified live vaccine could reduce the impact of the highly pathogenic PRRS wild type 1 virus. This is consistent with other researchers' findings [7][8][9].

Disclosure of Interest: None Declared

Keywords: Highly pathogenic type 1 PRRS virus, Ingelvac PRRS MLV, PRRS

Viral and Viral Diseases
PRRS

PO-PW1-172

Comparison of an in-house and a commercial ELISAs for antibody detection against highly pathogenic porcine reproductive and respiratory syndrome virus

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Introduction: Highly pathogenic porcine reproductive and respiratory syndrome (HP-PRRS) caused economic losses in swine industry. Several ELISAs have been developed for rapid diagnosis and assisting in control of the disease. The aim of this study was to determine an efficiency of a newly developed in-house ELISA for detection of antibodies against HP-PRRS virus (HP-PRRSV) in serum.
Materials and Methods: Nursery pigs from a PRRSV negative herd were randomly divided into 3 groups and housed in separate rooms. Group 1 (n=9) was a negative control. Group 2 (n=15) included pigs vaccinated with PRRS vaccine (Fostera™, Zoetis) at day 0 and experimentally infected with HP-PRRSV at day 28. Pigs in group 3 (n=15) were experimentally infected with HP-PRRSV at day 28. Sera were collected at day 0, 14, 28, 35 and 42. Real-time RT-PCR was used to detect the presence of HP-PRRSV RNA in serum. All sera were analysed with an in-house ELISA and a commercial ELISA (IDEXX PRRS X3 Ab test). Sera of group 2 at day 14, 28, 35 and 42 and sera of group 3 at day 35 and 42 were used to estimate sensitivity of the tests. The rest of the sera were used to evaluate specificity of the tests. Efficiencies of the tests were analysed by area under curve (AUC). Kappa coefficient and Pearson correlation coefficient between S/P ratios of sera of the two tests were calculated.
Results: Based on real-time RT-PCR, all pigs in group 1 stayed negative until the end of the study as well as all pigs in group 2 before vaccination and all pigs in group 3 before the challenge. The PRRSV were presented in 2 pigs in group 2 after vaccination whereas the viruses were detected in all pigs from group 2 and 3 after the challenge. Both ELISAs were able to detect seroconversion at 1 week after HP-PRRSV inoculation in either group 2 or group 3. The mean S/P ratio of group 2 from in-house ELISA was higher than that of a commercial ELISA after HP-PRRSV challenge (Fig 1). However, higher number of positive pigs was identified by the commercial ELISA after vaccination. Sensitivity and specificity of the in-house ELISA were 85.3% and 98.4%, respectively. Both ELISAs showed a good agreement based on kappa coefficient (k=0.8) and Pearson correlation coefficient (r=0.8). The AUC of the in-house ELISA was 0.94.
Conclusion: The newly developed in-house ELISA is reliable and can be used for antibody detection against PRRSV vaccinated and HP-PRRSV infected pigs. A sharp increase in S/P ratio after HP-PRRSV infection is a useful indicator for PRRS monitoring in a field situation.

Disclosure of Interest: None Declared

Keywords: ELISA, HP-PRRS

Viral and Viral Diseases
PRRS

PO-PW1-149

Efficacy of Ingelvac® PRRS MLV piglet vaccination in a large Korean farm

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Introduction: Recently most of the Korean swine farms use PRRS vaccine as a tool for sow herd stabilization against PRRS. However, perception to piglet vaccination is lacking so that infection by PRRSV and subsequent secondary bacterial infection still remains a problem especially after weaning. Moreover, in this 2-site managed farm, there are many economics losses in the fattener farm caused by PRRSV reinfection. In this study, we evaluated the efficacy of PRRS vaccination in piglets by measuring post weaning mortality and performance in nursery and fattening pigs.
Materials and Methods: This study was conducted in a 2,500 sow farm with a 2-site system. Mortality rate in the nursery is usually 1~2%, but shortly after arrival in the fattening farm, piglets are showing respiratory symptoms with reduced feed intake. In Sep'14, type 1 PRRS antigen was detected by PCR blood samples of 6 weeks age. After that, piglets showed neurological signs subsequently leading to death. To solve these problems, the farmer decided to vaccinate piglets at the age of 18 days with Ingelvac PRRS MLV using 3FLEX which is a licensed mixture of Ingelvac CircoFLEX®, Ingelvac MycoFLEX® and Ingelvac® PRRS MLV. This farm already used FLEXcombo, the licensed mixture of Ingelvac CircoFLEX® and Ingelvac MycoFLEX®. Within this study, 13 batches with 3FLEX vaccination (vaccinates) was compared to 13 batches with the original FLEXcombo vaccination only (controls). Monitored parameters were mortality in the nursery and performance of pigs in the fattening farm.
Results: From Aug to Oct 2014, 15,800 pigs (controls) were weaned from which 449 pigs died in the nursery. So in this period mortality rate was 2.84%. From Nov'14 to Jan'15, 14,507 pigs (vaccinates)were weaned from which 143 pigs died in nursery so that in this period mortality rate decreaded to 0.99%. In addition, we found that after 3 months of PRRS MLV vaccination the PRRS infection age was delayed from 6weeks to 9weeks. In the fattening farm, 1,074 pigs (controls) were dead or culled among 15,351. Mortality rate was 6.99%. Whereas 532 pigs (vaccinates) were dead or culled among 14,406. So mortality rate was decreased to 3.69%. ADWG was improved from 744g to 777g and FCR from 3.02 to 2.94. Antibiotics costs reduced from 23,173USD to 10,635USD. Average market days reduced from 190.4 to 186.3.

Conclusion: In this trial, we could demonstrate that, PRRS virus and secondary bacterial infection can be controlled by PRRS piglet vaccination. In addition, Ingelvac PRRS MLV provided cross protection against type 1 virus and with continuous vaccination we could delay infection age of wild type virus. Finally we could achieve much better production performance and economic benefit in fattener.

Disclosure of Interest: None Declared

Keywords: PRRS control, Cross protection, Fattener performance

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-097

Virulence of currently circulating PRRSV isolates under experimental conditions

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Introduction: In 2014 in North Carolina, anecdotal reports of a particularly virulent PRRSV isolate (RFLP cut pattern 1-7-4) were reported by practicing veterinarians. Reports indicated that the isolate was more transmissible, had higher levels of viremia for longer time periods and was more virulent in comparison to other circulating PRRSV strains. The objective of this study was to evaluate PRRS 1-7-4 isolates under experimental challenge conditions.
Materials and Methods: Sixty, PRRSV negative conventional, three week old pigs were randomized into four treatment groups (n=15/group). On D0, animals in Group 1 and 2 were inoculated intranasally with 2ml of one of two viral harvests derived from clinical samples collected in 2014 (RFLP pattern 1-7-4; lineage 1); animals in Group 3 were challenged with a positive control isolate (RFLP pattern 1-18-2; lineage 1); animals in Group 4 were inoculated with 1X PBS. Serum was collected on D0, 1, 3, 5, 7, 14, 28 and 35. On D14, 10 animals from each group were necropsied; remaining animals were necropsied on D35. At the time of necropsy, lungs were scored for macroscopic lesions and bronchoalveolar lavage was collected. Serum samples and BAL were tested by RT-PCR for the presence of PRRSV RNA and by ELISA for the presence of anti-PRRSV antibodies. BAL was cultured for the presence of bacteria.
Results: Least square mean lung lesion scores (with 95% confidence intervals) at D14 for Groups 1-3 were 3.1% (0.0–10.7%), 12.4% (5.6–21.3%) and 15.2% (7.3–25.3%), respectively. At D35, lung scores for Groups 1-3 were 2.8% (0.1% - 13.1%), 28.4% (3.9% - 63.8%) and 34.5% (1.3% - 82.6%), respectively. Viremia occurred in 13/15 animals in Group 2 and 3 by D1; all animals became viremic by D3. In Group 1, 13/15 animals were viremic by D3; all animals were viremic by D7. Viremia was detectable in all remaining challenged animals at D35. There was no significant difference in cycle quantification values among challenged animals. All challenged animals had detectable PRRSV antibodies by D14; animals challenged with placebo material remained seronegative throughout the trial. By D35, PRRSV antibodies were only detected in 0/5 and 1/5 pigs in Groups 1 and 2 respectively. Conversely, in Group 3, 4/5 animals had detectable PRRSV antibodies at D35. No viremia or seroconversion was detected in Group 4, nor were any lung lesions present.Additional results were compiled but not reported.
Conclusion: Two PRRSV isolates recovered in 2014 from farms experiencing severe clinical signs associated with PRRSV were compared to a virulent PRRSV isolate recovered in 2008. Based on lung lesions and viremia, the 2014 isolates were not more virulent in comparison to a 2008 isolate within the same lineage.

Disclosure of Interest: None Declared

Keywords: Isolate, PRRSV, Virulence

Viral and Viral Diseases
PRRS

PO-PW1-142

Effect of piglet vaccination against PRRS in Belgian farrow-to-finish herds

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Introduction: Porcine Reproductive and Respiratory Syndrome virus (PRRSv) may cause significant losses in pig herds due to pre-weaning mortality and reduced performance in growing pigs. In Belgium, the virus is widespread and endemic in most herds. An earlier trial showed that infection of PRRSv mainly occurs near the end of nursery (8-12 weeks of age (w)). PRRSv vaccines are effective under experimental conditions, but sometimes fail to cover the field expectations. Sow vaccination against PRRS is common practice in Belgian herds. Recently, also piglet vaccination strongly increased. The aim of this trial was to evaluate the serological response after vaccination and to investigate the effect of piglet vaccination on the timing of infection with PRRSv.
Materials and Methods: In 5 farrow-to-finish herds, with a PRRSv infection at the end of the nursery as shown by previous analyses, 1 batch of piglets was vaccinated with an attenuated EU PRRSv vaccine strain (Porcilis® PRRS, MSD, The Netherlands). Piglets were vaccinated by the farmer at 19-20 days of age, except for 1 herd in which piglets were 23-24 days old. In each herd, 60 piglets were randomly selected and individually earmarked (20 piglets from sows of parity 1, parity 2-4 and parity ≥5, resp). A horizontal serological screening was performed at 4, 7, 10, 13, 18 and 23w. Serum was examined for antibodies (ab) against PRRSv by IPMA and virus isolation was done on serum during seroconversion.
Results: In 3 herds, 62, 57 and 57% of piglets with a maternal ab (mab) titre of ≥160 at 4w seroconverted at 10w. In contrast, in 1 herd interference with mab was observed: 83% of piglets with a mab titre ≥160 did not seroconvert at 10w. Seroconversion indicated a PRRSv infection between 18 and 23w in those 4 herds, which was confirmed by isolation of wild type virus in 2 herds at 18w. The percentage of serological non-responding piglets to vaccination in the 4 herds was 50, 43, 47 and 33%, resp. In the 5th herd, viraemic piglets were found. Extremely high ab titres (≥2560) were detected at 4w and wild type virus was already isolated in piglets of 7w, indicating an early infection. Biosecurity was poor in the latter herd.
Conclusion: The results demonstrate that a substantial number of piglets do not seroconvert after vaccination. Piglet vaccination could not eliminate the virus from the herd, but seems to delay the timing of PRRSv infection towards the second half of the finishing period. Results underline that biosecurity measures play an important role in an effective control program. Further research is needed to investigate the clinical protection induced by vaccination, especially in the serological non-responders.

Disclosure of Interest: None Declared

Keywords: piglet vaccination, PRRSV, Serological evaluation

Viral and Viral Diseases
PRRS

PO-PW1-157

American PRRSv outbreaks in a traditional European strain PRRSv country. Case report.

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Introduction: Portugal is a country in south-west Europe where PRRSv strains affecting swine are mainly from the European type. Most of PRRS vaccines used have European strains. During late 2014 and 2015, we recorded 18 new cases of confirmed American type PRRSv (AS-PRRS), in farms previously positive for European type PRRSv (EU-PRRS) and vaccinated against European strains. All affected farms showed reproductive symptoms in sows and respiratory symptoms in nursery and fattening pigs. The purpose of this paper is to report this occurrence and to identify possible sources of infection for this new strain of PRRSv in our country.
Materials and Methods: We collected data from 20 farms from 4 Districts that had a confirmed PRRS outbreak (that means PRRS clinical signs and ELISA/RT-PCR positive for PRRSv).
Results: All farms belong to 4 different companies. Most farms (15/20) produce nursery piglets and 5 are farrow-to-finish. Clinical signs: 18/20 farms had mainly reproductive problems including late and mid-term abortions, low fertility and higher number of stillbirths, as well as lower vitality of piglets born with higher mortality during lactation stage (up to 25%). 8 farms had respiratory problems in nursery piglets and only 2 in fattening pigs. These farms were previously stable for ES-PRRSv, and were vaccinated with live vaccines. Materials collected for diagnosis included blood samples from affected sows, piglets and fatteners, lungs and abortions. Results for ELISA and/or RT-PCR confirmed AS-PRRSv strain in 18/20 farms. One strain was sequenced showing 99% homology with the US reference strain. Possible sources of the new virus: a) Live animals: All farms received negative AS-PRRSv gilts before; b) AI Semen: All farms received AI Semen from 3 external AI Centers, receiving boars from central Europe. PRRS control in the boars was done only by Elisa kits that may fail to detect AB from all PRRSv strains; c) Personnel working in several farms: Some maintenance workers did not follow strict biosecurity rules between farms, specially concerning tools and equipment; d) Lack of proper biosecurity of transport trucks: possible, but not different than before, one of the companies had high biosecurity protocols; e) lack of proper diagnostic tools in previous outbreaks: possible.
Conclusion: The origin of the AS-PRRSv that affects these farms could not be accurately identified. AI Semen is the most probable cause in at least 6 of the affected farms. Biosecurity failure from personnel and transport trucks are the 2nd and 3rd possible causes. Diagnostic failure to detect AS-PRRSv in the past is the 4th cause. Further sequencing of these PRRSv will enable us to understand the epidemiology of this outbreak.

Disclosure of Interest: None Declared

Keywords: AS-PRRSv, Portugal, PRRS positive boars

Viral and Viral Diseases
PRRS

PO-PW1-195

Sequence analysis of a novel PRRSV strain isolated in China

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Introduction: HP-PRRSV is a ubiquitous pathogen of Chinese pig farms and it has caused enormous economic losses to pig industry in China since it was detected in 2006. The main feature of HP-PRRSV was that it contained a noncontiguous 30-aa deletion in the highly variable region of NSP2. Many new PRRSV strains had been isolated in recent years, they had about 131-aa deletions that were identical to that in NADC30 and MN184 isolated in the United States, so these strains were called “NADC30-like strains”. In present study, we analyzed some gene sequence of GDSZ15, isolated in Shenzhen, with other PRRSV strains.
Materials and Methods: 25 tissue samples and the corresponding serum samples from the same piglet were collected from a pig farm in 2015. Severe respiratory symptoms were present in this farm. All samples were tested for viral RNA by reverse transcription PCR and primers specific for PRRSV open reading frame (ORF) 7. PRRSV isolation was performed in Marc-145 cells. Sixth-passage viral culture was used for sequence analysis. The highly variable region of the nonstructural protein 2 (Nsp2) and the entire ORF5 was amplified, then cloned into pMD-18T vector (TaKaRa, Japan). The clones were sequenced. Clustal W、DNA star and MEGA4 were used for sequences comparative analyses.
Results: It was found that ORF7 gene of PRRSV was detected only in 3 serum samples and 2 tissue samples. The 5 PRRSV-positive samples were used for virus isolation, and a strain of PRRSV (GDSZ15) was isolated from a serum sample. The sequence analysis showed that the highly variable region of NSP2 from GDSZ15 strain had 30-aa deletion while it had 130-aa deletion from the vaccine strain (TJM-F92) used in the pig farm. Comparative analyses of ORF5 sequences showed that GDSZ15 strain had 79.5% ,80.8%, 80.8%, 80.8%, 82.6% , 84.6% nucleotide identities and 82.9%, 82.9%, 82.9%, 82.0%, 84.4%, 84.0% amino acid identities with representative PRRSV strains (JXA1-R, HuN-F112, TJM-F92, VR2332, CH-1R and NADC30).
Conclusion: In the present study, we isolated a PRRSV strain from a pig farm in which the pig herd showed severe PCP clinical signs. All the analysis indicated that the isolated strain GDSZ15 was a new HP-PRRSV strain not the vaccine strain, and it was also not a NADC30-like strain.

Disclosure of Interest: None Declared

Keywords: PRRSV, Sequence analysis

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-107

Duration and persistence of PRRS ELISA antibodies in PRRS-infected and vaccinated sow herds during an eradication program with Porcilis®PRRS

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Introduction: To define breeding sow herds as PRRS non-suspect, PRRS ELISA negative results are required according to the German surveillance program. IDEXX X3 ELISA is a commonly used test because of its high specificity and sensitivity. To control and eradicate PRRSv, the choice of PRRS MLV vaccine is important with respect to spread of vaccine virus and impact on virus circulation. Only little is known about duration and persistence of PRRS-specific antibodies, measured by ELISA, in positive herds after withdrawal of MLV vaccination. Such information is important for planning of time schedules, replacement rates and economic outcomes of PRRS eradications programs.
Materials and Methods: The study was done in a multiplier farm with about 300 sows and 2000 piglets up to 11 weeks (partly multi-site production) and PRRSv (-) replacement gilts from an external source. The farm had a long term PRRS non-suspect status. The herd was infected with a European PRRSv isolate in march 2008. To control clinical symptoms of PRRSv, all sows (first step mass-vaccination, afterwards 50-70 days p.i.; 21.03.2008-12.03.2013) and piglets (age 14 days; 21.03.2008-24.07.2008) were vaccinated with Porcilis®PRRS (i.m.). Vaccination of the clinically PRRS stable herd was stopped in March 2013. The PRRS status was continuously checked with ELISA, PCR and introduction of PRRS ELISA negative gilts (sentinels). Twenty eight (28) months after last vaccination and 27 month after last introduction of vaccinated gilts, all sows and boars (n=307) were sampled and tested by IDEXX X3 ELISA and PCR (Virotype LDL; Tetracore).
Results: During the 28 month period, all sampled pigs (n=114) were PRRSv PCR (-). All piglets at the end of nursery (n=50) and sentinel gilts (n=52) were PRRS (-) in IDEXX X3 ELISA. The complete sampling of the sow herd on 17.07/24.07.15 showed PCR (-) results for all animals. Sentinel gilts (n=196) were ELISA (-), except for one sow (S/P 0.55). Seventy two (72) of the remaining PRRSv vaccinated sows (n=111; 64.9%) and 9 sows of the last group of PRRS vaccinated replacement gilts (n=17; 52.9%) were still PRRS ELISA (+).
Conclusion: The occurrence of a positive ELISA result in a non-vaccinated sow is in the range of the usual specificity (1/195; 0.5%). The high frequency (64.5%) of ELISA positive sows 28 months after the end of vaccination without reinfection is important for the planning of eradication programs. The results suggests that nearly all sows have to be replaced to achieve a PRRS ELISA negative status more quickly, which would be much higher than previously thought (40%).

Disclosure of Interest: None Declared

Keywords: Antibody persistence, PRRSv ELISA, PRRSv eradication

Viral and Viral Diseases
PRRS

PO-PW1-086

Developing Sampling Guidelines for Oral Fluid-Based PRRSV Surveillance

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Introduction: Oral fluids (OF) are a convenient surveillance sample because they are easily collected and can be tested for nucleic acids and/or antibodies for PRRSV and a variety of other pathogens. We are currently developing statistically-based guidelines for sample size, sampling frequency, and sampling location on a farm.
Materials and Methods: Two studies were conducted to map the spatial and temporal aspects of PRRSV infection and further the development of oral fluid sampling guidelines. **Study 1** - In one WTF barn on each of 10 production sites, OF samples were collected from 6 equidistant pens (~25 pigs per pen) every 2 weeks for 18 weeks. This provided a total of 600 OF samples. **Study 2** - In 3 wean-to-finish barns on one finishing site, OF samples were collected weekly from every occupied pen (108 pens; ~25 pigs per pen) for 8 weeks. This provided a total of 972 OF samples. OF samples were completely randomized and then tested for PRRSV RNA, IgG, and IgA. To date, statistical analyses have been done to examine spatial autocorrelation, compare detection based on systematic spatial vs random sampling, and compare sampling from the same pens vs alternate pens at each time point. Additional analyses are currently in progress.
Results: Analyses showed that the disease status of a pen in a barn was highly influenced by the disease status of other pens in the same barn. That is, the presence of ≥ 1 positive pens increased the probability of another positive pen (Table 1). The probability of a positive test result increased as the number of positive pens in the barn increased, but this was more true for ELISA than RT-PCR. For most swine veterinarians, the spatial relationship in disease status is obvious, but spatial autocorrelation has previously not been quantified at the barn level and has important implications for surveillance.

Analysis has also showed that systematic spatial sampling was as good as or better than random sampling. Sampling the same pens at each time point was more effective than changing the pens sampled at each time point. "Systematic spatial sampling" has not been explored extensively in veterinary surveillance, but has been used extensively in other fields of study.
Conclusion: The analyses performed to date showed that systematic spatial sampling is a viable approach for routine surveillance. Sample size calculations are in progress, but frequency of sampling is more important than sample size. That is, fewer samples collected routinely are more useful than more samples collected infrequently. Overall, the results suggest that a simple, but reliable, oral fluid-based sampling strategy can be developed.

Disclosure of Interest: None Declared

Keywords: Epidemiology, Oral fluids, Sampling

Viral and Viral Diseases
PRRS

PO-PW1-163

Value of Gilt Acclimation to PRRS

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Introduction: PRRS infection of breeding herds represents a global opportunity to improve animal well-being and farm profitibility. The annual loss from PRRS in U.S. breeding herds is estimated at $302.06 million. The majority of this loss is due to reduced revenue from weaning fewer pigs. With an estimated 23-41% annual PRRS breeding herd infection rate there is tremendous value in gilt acclimation which decreases novel PRRS infection impact through improved weaned pig output.
Materials and Methods: The value of gilt acclimation for PRRS is determined by comparing the weaned pig output as a percentage of baseline in acclimatized versus non-acclimatized herds infected with a common PRRS virus. Baseline herd weaned pig output is calculated as the mean weaned pig output from each individual herd during the 52 weeks preceeding novel PRRS infection. Performance relative to baseline post-infection is described as the percentage of the baseline weaned pig output acheived each week following novel PRRS infection. Non-acclimatized herd performance versus acclimatized herd performance is assessed by comparing the weighted mean percentage of baseline weaned pig output achieved in farms infected with a common PRRS virus. Value of gilt acclimation for PRRS is then defined as the overall revenue opportunites created by additional weaned pig output from acclimatized herds using a standard value of $40.00 per weaned pig. The value of gilt acclimation informs a PRRS infection rate at which herds should or should not be acclimatized for PRRS.
Results: In the 10 weeks following a novel infection, acclimatized and non-acclimatized herds weaned 94% and 55% of their baseline pig volumes, respectively. Assuming an average weaned pig volume of 0.5 weaned pigs per breeding female per week this production improvement will result in 2 more pigs weaned per breeding female, an opportunity value of $80.00 per breeding female. Additional performance data is being collected to value acclimation subsequent to the first 10 weeks of infection. These final acclimation values will be available and presented at the meeting to estimate the PRRS infection rate at which herds should or should not be acclimatized for PRRS.
Conclusion: Improved control of novel PRRS infections in breeding herds represents a remarkable improvment opportunity for animal health and farm profitibility. Regardless of the acclimation strategy employed, PRRS acclimation results in more pigs weaned in the face of a novel PRRS infection. Comparing the performance of acclimitzed herds versus non-acclimatized herds infected with a common virus allows producers and veterinarians to determine the need for acclimation in their herds.

Disclosure of Interest: None Declared

Keywords: Acclimation, gilt, PRRS

Viral and Viral Diseases
PRRS

PO-PW1-124

Surfactant Proteins Expression in Lung Injury by Porcine Reproductive and Respiratory Syndrome Virus Infection

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Introduction: The porcine reproductive and respiratory syndrome virus (PRRSV) cause lung injury in the infected pigs. The alveolar type II epithelial cells synthesize and secret the lipoprotein compounds, pulmonary surfactants, to reduce the alveolar surface tension and prevent alveolar collapse. While the hydrophobic surfactant proteins maintain the alveolar surfactant balance, the hydrophilic surfactant proteins participate in the lung's innate immunity. Surfactant proteins are also important for postnatal lung development and prevention of infection caused lung injury. How PRRS infection affects surfactant protein expression is not clear.
Materials and Methods: In this report, using quantitive PCR, we examined the expression patterns of four surfactant proteins in the postnatal developmental pig lungs and in the lungs of breed pigs infected with porcine reproductive and respiratory syndrome virus (PRRSV).
Results: Comparison of SPs during the day before and after the weaning, we found that SP-A, SP-B, and SP-C were slightly affected by weaning, and but the SP-D expression were dramatically decreased by weaning. The later postnatal development, while SP-D expression was remained as the same as that at the day of weaning, the expression of SP-A and SP-C were increased and the expression of SP-B started to decrease. In the PRRSV infected swine lungs, the infection leaded to an increased expression of SP-B and SP-D and a great decreased expression of SP-C.
Conclusion: Our results suggest that differential expression of surfactant proteins may be important for postnatal lung development and that unbalanced expression pattern of SP induced by virus infection may contribute to the lung injury.

Disclosure of Interest: None Declared

Keywords: lungs, prrs, surfactant proteins

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-161

The effects of using attenuated PRRSV vaccine in sows on the distribution of PRRSV, PCV2, and Mhp in their offsprings

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) is always the first devastating disease in Taiwan pig farms. Co-infection of the virus with other respiratory pathogens exaggerates the outcome of PRRSV-associated porcine respiratory complex (PRDC) and causes a lot of economic impacts on farmers. The aim of this study was to compare the PRRSV-specific antibodies and pathogenic loads in serum of pigs born from sows with high (S/P ratio: 1.51-3.25) or low antibodies (S/P ratio: 0.41-1.17) against PRRSV and with immunization of PRRS modified live vaccine (Ingelvac PRRS® MLV).
Materials and Methods: There are totally 60 sows evenly distributed into four groups: sows with high antibodies and low antibodies against PRRSV (30 sows each) with or without PRRSV vaccination. So, each group contained 15 sows and half of these four groups were immunized with vaccine (Ingelvac PRRS® MLV) between 76 – 84 days of gestation (HV/LV), and the other half acted as control (HC/LC). Then, 80 4 week-old pigs randomly selected from each group were used and blood samples were collected at 4, 6, 8, 10, 12 week-old. Quantitative real-time polymerase chain reaction (qrt-PCR) was employed to quantify the levels of PRRSV, porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* (Mhp), and Enzyme-linked immunosorbent assay (ELISA) to detect the antibodies against those three pathogens.
Results: The main finding indicated that piglets born from sows with high PRRSV antibodies (HV/HC) showed better average titers (S/P values, 0.5-1.16) and positive rates (50-95%) of PRRSV antibodies at 4-, 6- and 8- week-old pig than their counterparts. In 6-week-old pigs, the viral loads of PRRSV in group H-Con and H-V were lower than others ($p \leqq 0.05$) , whereas there was no significant difference in pathogenic loads of PCV2 and MHYO among groups.
Conclusion: Based on the results, sows with high titers of PRRS antibodies with or without vaccination offer their offsprings a better level of ELISA antibodies which somehow provide a better protection against PRRSV infection as seen on their clinical growth performance, especially for pigs during the postweaning stage. It is also important to note that other means of preventing measures should be also implemented to ensure this protection not be interfered by other pathogens and un-appropriated management.

Disclosure of Interest: None Declared

Keywords: PRDC, PRRSV , PRRSV Live Vaccine

Viral and Viral Diseases
PRRS

PO-PW1-125

Oral Fluid sampling as a tool for PRRSV surveillance in gilts.

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Introduction: Gilts are a potential source of porcine reproductive and respiratory syndrome virus (PRRSV) and their introduction into breeding herds is a pivotal step of disease control. Data on the onset of PRRSV infection in replacement gilts during the acclimatization period would be useful for PRRS control strategy. This study was aimed to compare PRRSV detection and PRRSV-specific antibody responses in Oral Fluid (OF) versus serum samples in order to determine whether pen-based OF sampling could be used as a tool for PRRSV surveillance in gilts in breeding herds in Italy.
Materials and Methods: The study was carried out in 11 breeding farms endemically infected with PRRSV in a high pig density area in Italy. Sows had been vaccinated with PRRSV modified-live virus and inactivated vaccines in 4 and 2 farms, respectively. No vaccines were used in gilts in the other 5 farms. OF was sampled in 3 pens with 5 animals each. Those gilts were also bled individually to obtain serum samples Samplings were performed at the beginning of the acclimatization period (T0) , at 4 (T1) and 8 weeks (T2) later. Sera and OF were used in a PRRSV real-time quantitative reverse transcription polymerase chain reaction (qPCR) and a PRRSV-specific serum antibody ELISA. PRRSV IgA and IgG Ab assays were used only in OF A total of 495 sera and 99 OF samples were analyzed.
Results: In one out of 11 herds, all the gilts were completely PRRSV-negative by qPCR, and Ab-negative in the assays on serum and OF samples at the three time points. In the other 10 farms, animals showed PRRSV infection At T2, serum and OF samples of the 10 farms were Ab-positive, only 6 of them being also qPCR- positive. IgA and IgG Ab assays on OF were negative at T0 and positive at T2 in 7 and 3 farms, respectively. The concordance of qPCR results on OF vs sera was 96.9%, 93.9% and 69.7% at T0, T1 and T2, respectively. At T2, in 7 samples qPCR was positive in OF and negative in sera. The concordance of OF vs serum Ab assays was 87,9 % at T0, 84.8 % at T1 and 93, 9% at T2. A comparison of antibody responses in qPCR-positive vs. negative oral fluid samples showed higher S/P ratios in qPCR-positive oral fluid samples (mean S/P 4,18 vs. 3,67).
Conclusion: The concordance of qPCR and antibody ELISAs in gilts was high at all the time points (T0, T1 and T2). The prevalence of PRRSV qPCR positive samples in OF was greater when compared with serum at T2. Although the approach should be validated in further field trials, the results of this study showed that gilt oral fluid samples could provide an efficient and sensitive approach to PRRSV surveillance in infected or presumed-negative pig breeding herds.

Disclosure of Interest: None Declared

Keywords: PRRSV, Oral fluid, surveillance

Viral and Viral Diseases
PRRS

PO-PW1-117

PRRS antibody ELISA response in serum of piglets after PRRS MLV vaccination

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Introduction: Veterinary practitioners in the Netherlands often try to use PRRS antibody titers as a tool for compliance of modified-live (MLV) PRRS vaccination in piglets, or to show PRRS field virus infection in piglets regardless of previous PRRS MLV vaccination.
The objective of this field study was to evaluate results of PRRS antibody testing in the serum end of nursery, at 5-7 weeks after PRRS MLV vaccination.
Materials and Methods: In five commercial Dutch sow herds, piglets were PRRS MLV vaccinated (PRRSFLEX EU®) at 3-5 weeks of age. At 5-7 weeks after vaccination, when the piglets were 9-10 weeks of age, at each farm piglets were bled and the samples tested for PRRS antibodies (IDEXX PRRS X3 Ab Test ELISA).
Results: At four farms 30 samples were tested, in one farm 14 samples were tested. The percentage of antibody positive samples per farm (S/P ratios above 0.4) varied between 50 and 97%. The average S/P ratio per farm varied between 1,00 and 2,29. The Standard deviation of the S/P ratio per farm varied between 0,67 and 1,18.
Conclusion: In every farm the percentage of ELISA positive samples was below 100%, one farm had 50% of so called 'non-responders' (negative samples). As PRRS protective immunity is based upon neutralizing antibodies and/ or cellular immunity, finding 'non-responders' cannot be regarded as proof of lack of immunity.
Remarkable is the variation (standard deviation) in S/P ratios per farm and between farms. This makes drawing conclusions on the average S/P ratios questionable. This will be even more difficult when testing a low number of samples per batch, e.g. to test 5-10 samples per batch, as the variation will have a strong at random effect on the results.
ELISA kits are useful for the detection of antibodies against either genotype of PRRSV, but cannot discriminate antibodies against vaccine virus from field virus. So no conclusions can be drawn on that.
We conclude that the use of a commonly used PRRS antibody ELISA kit does not provide any information on how to answer the following questions: a) have the piglets been properly PRRS MLV vaccinated and b) is there proof of PRRS field virus infection in PRRS MLV vaccinated piglets

Disclosure of Interest: None Declared

Keywords: Compliance, ELISA, PRRS MLV vaccine

Viral and Viral Diseases
PRRS

PO-PW1-137

Comparative efficacy evaluation of two modified-live PRRS vaccines

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Introduction: The implementation of a systematic methodology for PRRS control that utilizes modified-live vaccine (MLV) for the control of wild type-PRRSV infections can mitigate the consequences of infection on health and performance. It is necessary for MLV to be effective against heterologous challenge with current PRRSV field isolates. The objective of this study was to directly compare the efficacy of Ingelvac PRRS® MLV (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) and Prime Pac™ PRRS+ (Merck Animal Health, Omaha, NE) against a heterologous PRRSV challenge.
Materials and Methods: The study was performed in seventy, PRRS naïve three-week-old pigs. On Day 0, Group 1 (n=20) was vaccinated with Ingelvac PRRS® MLV (2 ml IM per label). Group 2 (n=20) was vaccinated with Prime Pac™PRRS+ (1 ml IM per label). Group 3 (n=20) was a non-vaccinated challenge control group (NVC). Group 4 (n=10) was an Ingelvac PRRS® MLV vaccinated, non-challenged group. On Day 28, Groups 1, 2 and 3 were challenged intranasally with 2.0ml containing 4.1log$TCID_{50}$/ml of virulent PRRSV SDSU-73. On Day 42, 10 pigs were selected from each of Groups 1-3, necropsied, and lung lesions scored. The study was terminated on Day 70 and the remaining pigs were necropsied and lung lesions scored. Blood was collected from all pigs to assess the serologic response and viremia (Quantitative PCR; BIVI HMC Ames, Iowa). Rectal temperatures and ADWG were also measured and evaluated.
Results: Both vaccinated groups demonstrated a large reduction in PRRSV associated lung lesions (Group 1, 0.5%; Group 2, 1.3%) compared to NVC (28.6%; median lung lesion). From day 42 to 63, Group 1 demonstrated fewer percent PCR positive pigs than the Group 2 and NVC pigs. The reduction of viremia following challenge occurred earlier in the Group 1 compared to the Group 2, and the pattern of viremia reduction in Group 2 was similar to the NVC. Group 1 maintained lower average temperatures throughout the challenge phase (days 28-42) compared to Group 2 and challenge control group. Between days 28 and 70, Group 1 had a 17% higher ADWG (1.41 lbs/day) when compared to both the Group 2 (1.17lbs/day) and the NVC (1.18lbs/day). Group 4 (vaccinated, non-challenged) demonstrated the best ADWG across treatment groups (1.67 lbs/day).
Conclusion: Virulent PRRSV challenge has a biologic impact as measured by increased temperature and viremia and their influence on ADWG. This study is another example demonstrating the ability of modified-live PRRS vaccines to protect against a relevant PRRSV challenge. Implementing vaccine in a systematic methodology for PRRS control can mitigate the consequences of infection and subsequently improve the health and performance of pigs.

Disclosure of Interest: None Declared

Keywords: Comparing, Efficacy, Ingelvac PRRS MLV

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-138

TIME TO PRRS STABILITY USING A COMERCIAL MLV VACCINE

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Introduction: PRRS virus is one of the most important diseases worldwide. The cost of an outbreak in the sow herd is around 255$. The objective of this study is to evaluate the time to stability (TTS), which means time to produce negative pigs at weaning age after a PRRSv outbreak in a multi-sites production system.
Materials and Methods: The study was conducted in a 4,900 sow farm, located in Jalisco, México, who broke with PRRSv at week 35 of 2014. The sow herd was vaccinated every 4 months with Ingelvac® PRRS MLV and the last mass vaccination was in week 37. Prior to the 2014 outbreak the farm was considered positive stable (Category II-A).
The diagnostic protocol was 30 aborted sows (100% positive), and 28 piglets at weaning age (88% positive), using serum samples by qRT-PCR PRRSv and RFLP; the boar stud was positive for PRRSv by ELISA (97% positive). The main action plan was to depopulate the boar stud and use free PRRSv semen from other source, also a load, close and homogenize program (herd closure for 210 days), and stablish mass vaccination against PRRS with Ingelvac® PRRS MLV for Sows and replacement gilts. The main goal of the intervention protocol was to control the field isolation. The system will maintain Ingelvac® PRRS MLV every 3 months.
The mass vaccination protocol with Ingelvac® PRRS MLV was implemented in week 1 of 2015 and the sows were revaccinated 4 weeks later. We established a monitoring protocol to evaluate TTS. 90 nursery piglets were tested by qRT-PCR PRRS, at 12, 16, 20, 24 and 28 weeks using pools of 5.
Results: Using RFLP we found that the PRRSv cut pattern was 1-6-3. After a herd closure and mass vaccination the 5 consecutive monitoring tests in piglets were negative by qRT-PCR. TTS demonstrate the breed to wean stabilization.
Conclusion: The load-close-homogenize (LCH) program for multisite systems is one of the most effective management against PRRSv. In this study after 24 weeks of management and immunization protocols, the farm changed the status to a Category II-A. We confirmed the lack of viremia in weaning age pigs and no clinical signs of PRRSv in the breeding herd after a 90 day period. Different studies prove that the use of the Ingelvac® PRRS MLV vaccine not only reduce the time to baseline production (TTBP) but can also improve the TTS.

Disclosure of Interest: None Declared

Keywords: PRRS MLV vaccine, PRRS outbreak, Sow herd stability

Viral and Viral Diseases
PRRS

PO-PW1-184

Field observation: No adverse reactions after PRRS EU mass vaccination of sows

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Introduction: Because of presumed side effects, Dutch farmers are often unwilling to vaccinate sows in first month and in the last weeks of gestation. In 2015 a new PRRS vaccine for sows is introduced for the Dutch market (ReproCyc® PRRS EU, *Boehringer Ingelheim*), which is recommended to use in 3 to 4 mass vaccinations of the sows per year.
This is an evaluation of adverse reactions after mass vaccination of sows with ReproCyc PRRS EU under field circumstances.
Materials and Methods: In a Dutch 1300 sow herd with a weekly rhythm, pregnant sows are housed in 3 dynamic groups of 350 sows. Sows enter the groups within 5 days after insemination.
ReproCyc PRRS EU mass vaccination took place at 30 June 2015 and 5 October 2015. For one week before to one week after the October mass vaccination daily records were kept of sows that did not consume their daily feed ration at the Electronic Sow Feeders (ESF) (Manibeck, Pigtek).
Parameters obtained from the management system (Agrovision) were: percentage of re-breeders (weekly) from 4 weeks before to 4 weeks after mass vaccination, and the percentage of pre-weaning piglet mortality per month (year to date).
Results: After the mass vaccinations no adverse reactions were seen by the farmer and his staff.
The percentage of sows that did not eat their daily ration varied from 0,2 to 1,1%, with 0,5% on the day of vaccination and 0,3% the day after.
The percentage of re-breeders per week varied between 10,0 and 22,5%, in the 4 weeks before and 4 weeks after mass vaccination at 5 October the average was 17,1% and 15,6% respectively.
Pre-weaning mortality varied from 7,1 to 10,2% per month, in the month following mass vaccination this was 8,8% and 8,4% respectively.
Conclusion: Any effects on feed intake can be expected within two days after vaccination, this was not observed after mass vaccination.
We assumed that effects of re-breeding could be expected in the weeks following vaccination. No increase of re-breeders was observed.
When sows do not feel well we assumed the sows to have more problems fostering their piglets, leading to an increase in pre-weaning piglet mortality. No increase of mortality after vaccination could be observed.
The results of this evaluation show a good safety of ReproCyc PRRS EU used in mass vaccination in sows, which is in line with other studies.

Disclosure of Interest: None Declared

Keywords: mass vaccination, PRRSV Live Vaccine, safety

Viral and Viral Diseases
PRRS

PO-PW1-191

The impact of concurrent PRRSV, PEDV, and Salmonella health challenges on nursery pigs

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) and porcine epidemic diarrhea (PED) are economically significant viral diseases that impact pig production and wellbeing worldwide. These viral infections may increase likelihood and impact of secondary enteric bacterial infections, including *Salmonella*. Our objective was to determine the extent to which PRRS and/or PED alters nursery pig performance and intestinal pathology when subsequently challenged with *Salmonella typhimurium* (ST).
Materials and Methods: Two replicates of 42 and 40 gilts, respectively, naïve for PRRS and PED, were assigned to one of eight treatments. Pigs were penned in pairs with each treatment in separate rooms to avoid cross contamination. Treatments included: 1) Control (n=7 pens), 2) PRRS only (n=6 pens), 3) PED only (n=6 pens), 4) PRRS+PED (PRP, n=6 pens) 5) ST only (n=4 pens), 6) PRRS+ST (n=4 pens), 7) PED+ST (n=4 pens) and 8) PRP+ST (n=4 pens). Treatments 2, 4, 6 and 8 were inoculated with live PRRS virus on day zero (D0). Treatments 3, 4, 7 and 8 were inoculated with PED virus on D14. Treatments 5, 6, 7 and 8 were inoculated with ST on D19. Pen performance (ADG, ADFI and G:F) was recorded and calculated over a 21 day period and all pigs were euthanized on D21. Jejunum and ileum were collected at euthanasia and evaluated for villus atrophy and inflammatory infiltrates and assigned a composite lesion score. Serum QPCR was used to confirm infection with PRRS. Fecal QPCR was used to confirm infection with PED and ST. Data were analyzed in a 2^3 design to determine main effects and interactions between diseases.
Results: Control pigs remained PRRS and PED negative and did not shed ST throughout the study. Compared to Control pigs, PRRS, PED and PRP significantly reduced ADG and ADFI ($P < 0.01$); however, in the 2 days of ST infection, there was no significant ST or treatment by ST interaction. There was a tendency ($P = 0.065$) for a treatment by ST interaction on G:F, while the main effect of ST ($P < 0.01$) reduced G:F. There was a significant interaction for ST to increase composite lesion scores ($P < 0.01$). The inclusion of ST resulted in almost a 2-fold increase in composite lesion scores for PED and PRP. Interestingly, when ST was added to PRRS alone, lesion scores increased more than 3-fold ($P < 0.01$) compared to ST alone.
Conclusion: In summary, pigs exposed to PRRS and/or PED virus and subsequently challenged with ST had increased severity of lesion scores as well as greater negative impact on pig performance compared to viral challenges alone. This suggests concurrent viral and bacterial infections may have synergistic adverse effects on nursery pig health beyond the expected sum of individual infections alone.

Disclosure of Interest: None Declared

Keywords: PED, PRRS, Salmonella Typhimurium

Viral and Viral Diseases
PRRS

PO-PW1-112

PRRS eradication on a newly infected 3000 sow farm using MLV and KV

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Introduction: A 3000 sow SPF GGP farm has been infected by PRRS virus in 2014 august with severe clinical signs. The eradication process has started in 3 month.
Materials and Methods: The eradication has been done by herd closure on the sow farm and partial depopulition on the rearing farm. Strong internal biosecurity measures were introduced on the farm. The farm started 3week batch system to help to clear the virus from the farrowing rooms and to make AIAO in the nursery by building. The herd has been vacinated by MLV (Unistrain) 2 times on week 40 and 44 in 2014. The piglets before weaning (at 3 weeks of age) have been monitored by PCR (180 samples/batch) pooled by 5) form the begining of 2015. At the first sampling we have checked the serology titers too. The third of the 3 week old piglets were seronegativ on the 1st week of January so an inactivated vaccination (Progressis) has been implemented 3 weeks prior farrowing to raise the maternal immunity. In late April more then 90% of the weaned piglets were PCR positiv without any clinical signs so in May we have made a mass vaccination again by MLV (Unistrain).
Results: The KV significantly increased the maternal antibody titers.
The first negativ batch has been weaned at the end of June 2015 and so far all the batches are negativ. In the rearing facilities all the piglets are negativ till the end by now.
Conclusion: The eradication is possible by herd closure even on a large farm using MLV for the mass infection.
The KV is a good tool to raise the maternal immunity after MLV vaccination.
Even a large farm can be managed by 3week batch system.

Disclosure of Interest: None Declared

Keywords: Eradication, MLV, PRRSV

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-143

Evaluation of porcine reproductive and respiratory syndrome virus (PRRSV) challenge dose in vaccinated pigs

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Introduction: The swine industry continues to experience significant losses due to PRRSV infections. The infectious dose of PRRSV has been shown to be very low, therefore highly infectious. The objective of this study was to evaluate the effect of PRRSV challenge dose in vaccinated pigs.
Materials and Methods: The study was performed in ninety, three-week-old pigs from a PRRS naïve and PCR negative source. Groups 1-5 (n=10) were vaccinated (Day 0) with Ingelvac PRRS® MLV (2ml IM). Forty pigs served as non-vaccinated challenge controls (NVC-Groups 1-4; n=10 per group). Groups 1-4 were challenged on Day 28 intranasally with 2.0 ml of virulent PRRSV SDSU-73 at 4log, 3log, 2log or 1log$_{10}$TCID$_{50}$/ml, respectively. Group 5 was not challenged. Temperature (Day 28-42), viremia and ADWG (Day 28-70) were evaluated and statistically analyzed.
Results: At all challenge doses, Ingelvac PRRS® MLV vaccinated pigs demonstrated a significant decrease in days pyrexic compared to NVC groups ($P<0.05$). At PRRSV challenge doses of ≤2logs, the average temperatures of the vaccinated challenged pigs were similar to Group 5. As compared to the NVC, there was a significant increase in ADWG ($P<0.05$) in the 3, 2 and 1log groups, and at $P<0.07$ in the 4log group. The ADWG of vaccinated groups challenged with ≤2logs of PRRSVwas numerically similar to the ADWG of Group 5. There was a measurable negative impact on ADWG in the NVC groups with no difference across all challenge doses. Ingelvac PRRS® MLV vaccinated pigs demonstrated fewer percent PCR positive pigs than NVC pigs at all challenge doses. As the challenge dose decreased the percentage of viremic pigs in the vaccinated groups decreased, with viremia in vaccinated pigs challenged with ≤2logs of PRRSV similar to Group 5. At all challenge doses, the NVC pigs show similar post-challenge viremia profile.
Conclusion: In this study, at all challenge doses, Ingelvac PRRS® MLV vaccinated pigs demonstrated mitigated biological consequences of a relevant PRRSV infection, with a reduction in post-challenge viremia, temperature and increased ADWG as compared to NVC pigs. For all endpoints, minimal difference between 0, 1 and 2log challenge in vaccinated animals indicates a challenge dose effect. Based on challenge dose (≤2logs), the consequences in vaccinated pigs were similar to non-challenged pigs. The post-challenge viremia and ADWG of NVC pigs were similar across all challenge doses, indicating no challenge dose effect and a measurable impact in unvaccinated pigs. Implementation of vaccine along with the other components for PRRS control can mitigate the consequences of PRRSV infection subsequently improving health and performance.

Disclosure of Interest: None Declared

Keywords: Challenge Dose, Ingelvac PRRS MLV, Vaccination

Viral and Viral Diseases
PRRS

PO-PW1-085

Factors that influence mechanical transmission of Porcine Reproductive and Respiratory Syndrome Virus at Slaughter Plant Lairage

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRS) can decrease growth and cause infertility and abortion in adult pigs. This study was conducted to further understand the risk associated with PRRS transmission/movement at the lairage facility.
Materials and Methods: A contact model for the unloading dock was employed using a 68L plastic tub. The model dock was contaminated with a mixture of 1L of PRRS and PEDV-negative manure and 1L of new pine shavings. This material was mixed with 10cc of Ingelvac PRRS MLV (Boehringer Ingelheim Vetmedica Inc). The foot contact was modeled by using a clean plastic boot cover to step from the model dock onto a model trailer (40.6cm x 29.2 cm, 7000-45 Disposable Aluminum Cookie Sheet). Samples were collected using a method previously described by Lowe et al. (2014). In experiment 1, 32 replicates of each of the temperatures on the model trailer (4°C, 15°C or 28°C) from the model dock prior to contact, immediately and 60 minutes after contact. In experiment 2, a 2 x 2 x 2 arrangement was used to assess the effects of temperature (4°C, 32°C), UV Light (Ambient/Supplemental light), and mechanical scraping (De-bulked/not De-bulked) on PRRS RNA transfer. Temperature was achieved with either an ice bath (4°C) or a heat lamp (32°C). A UV bulb was placed 60 cm above the floor of the tub. De-bulking was achieved by dumping the material out of the tub to simulate the act of scraping the dock. Samples were collected at 0, 10 and 60 minutes. Samples were sent to Iowa State University Veterinary Diagnostic Laboratory for rtPCR PRRS RNA analysis. All data were analyzed using Statistix 10.0.
Results: Temperature in the trailer did not affect PRRS RNA recovered 60 minutes ($p=0.36$). If PRRS RNA was detected on the model dock, PRRS RNA was transferred and detected on the model trailer 80% (95% CI 70.0%, 90.0%) of the time. Only de-bulking reduced the risk of transfer (OR=0.14 95% CI [0.06, 0.32], $p<0.001$). Hot temperatures on the dock increased the risk of transfer (OR=2.7 95% CI [1.43, 5.10], $p=0.001$). Time from dock contamination to the contact event was not associated with any changes in amount or transfer rate.
Conclusion: These data suggest that contact at the harvest plant lairage is a risk factor for PRRS RNA transmission between sites when inadequate hygiene is practiced on livestock trailers. These risks can be mitigated, but not eliminated through mechanical removal of gross contamination of the dock. Further work is needed to validate these data under field conditions and to model the impact of a risk reduction of this magnitude on PRRS transmission risks at the industry level.

Disclosure of Interest: None Declared

Keywords: Lairage, PRRSV, Transmission

Viral and Viral Diseases
PRRS

PO-PW1-082

Evaluation of agreement between serum and oral fluids to investigate PRRS in weaning pigs

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Introduction: The use of oral fluids as a sample in swine diagnostics has already proved to be a reliable source of information for the detection of both antibodies and etiological agents (Prikett 2008).
Oral fluids are not yet diffusely used; in fact many laboratories have not validated protocols or kits to test this particular matrix and there is still some criticisms about the efficiency of this material.
The aim of our study was to compare the use of serum with oral fluids as a sample for the detection of PRRS virus and antibodies in weaned pigs of different ages, in order to better evaluate the correspondence of the two materials to investigate the presence and diffusion of PRRS.
Materials and Methods: We selected ten reproduction farms. In each farm we collected an oral fluid sample and 5 serum samples from a box for each age of weaned pigs, the youngest animals being around one month and the oldest around three months old.
At first a cotton rope was hanged up and left available to the animals of each selected box for 20 minutes before oral fluid samples were collected and refrigerated. Subsequently, 5 animals from each selected box were randomly chosen for the collection of a blood sample.
Each oral fluids sample and a pool of five serum samples, prepared according to the box partition of animals, were tested by PCR for the presence of PRRSV. Individual samples of oral fluids and serum were tested for antibodies using the kits IDEXX PRRS OF Ab test for oral fluids and IDEXX PRRS x3 Ab test for serum samples.
Results were statistically analyzed to evaluate agreement between the two different matrixes calculating the Cohen K value for each age group.
Results: 47 oral fluid samples and 235 serum samples were collected from 10 different farms and included in the study.
29/47 oral fluid and 28/47 pooled serum samples tested positive for PRRSV by PCR.
42/47 oral fluids and 105/235 serum samples tested positive for the presence of PRRS antibodies and, according to the box partition of animals, 40/47 boxes had at least one animal positive for antibodies.
The Cohen K value was considered excellent for groups of 70 and 90 days of age (K=1), and good for groups of 30 (K=0,80).and 50 days (K=0,77).
Conclusion: The comparison between these two types of samples showed a good agreement for both positive and negative results, with a better reliability as animals became older. This is probably connected with the enhanced exploration behavior with increasing age.
Oral fluids proved to be a reliable sample and a good substitute to serum as a screening strategy to detect the presence and diffusion of PRRSV in groups of animals older than 30 days.

Disclosure of Interest: None Declared

Keywords: Oral fluids, PRRS, weaning pigs

Viral and Viral Diseases
PRRS

PO-PW1-133

A multiplexed immunoassay for simultaneous detection of antibodies to PRRSV, Actinobacillus pleuropneumoniae and Salmonella in pigs

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Introduction: To offer routine diagnostic analyses that are cheaper and less time-consuming than our current in-house ELISAs, we have developed and evaluated a Luminex-based multiplexed immunoassay that facilitates simultaneous detection and distinction between antibodies to porcine reproductive and respiratory syndrome virus (PRRSV) Type 1 and Type 2, *Actinobacillus pleuropneumoniae (App)* serovars 2, 6 and 12 as well as *Salmonella Typhimurium* and *Salmonella Choleraesuis*.
Materials and Methods: The multiplexed immunoassay employs the same antigens that are used for our in-house ELISAs, including crude viral lysates of PRRS strains as well as purified bacterial lipopolysaccharides from *App* serovars and *Salmonella* subtypes. Antigens were coupled separately to seven batches of magnetic beads with variant internal fluorescence, plexed and incubated with serum from infected or non-infected pigs. Bound serum antibodies were detected with layers of biotinylated anti-pig antibodies and fluorescently labelled streptavidin. Samples were analyzed in a Bio-Plex 200 reader, results were read as median fluorescence intensities and a sample-to-positive ratio was calculated. Receiver Operator Characteristic (ROC) curve analysis was used for comparing the quality of the assay with our in-house ELISAs.
Results: Converting individual ELISAs with different assay conditions into a single multiplex analysis introduces the challenge of defining optimal assay conditions that can be applied to all antigen-antibody interactions included. By testing various assay conditions such as reactant concentrations, temperature, reaction time and buffer composition in singleplex assays, we succeeded in defining optimal assay conditions that could be applied to all analytes. Antigen-specific reactivities measured in a singleplex format were retained when combining beads coupled with antigens from the various pathogens in a multiplex format, indicating limited cross-reactivity. When validating the multiplex assay with a large number of sera from infected and non-infected pigs we observed a good correlation with our in-house ELISAs.
Conclusion: A bead-based multiplex assay was designed to simultaneously detect and distinguish antibodies in a single serum sample towards Type 1 and 2 strains of PRRSV, *App* 2, 6 and 12 as well as *S. Typhimurium* and *S. Choleraesuis*. The assay has high sensitivity and specificity for detection of antibodies in pig serum to all agents included and shows good overall agreement with our well-established in-house ELISAs. We expect that implementation of multiplex analysis in routine serological diagnostics in pigs will lower the cost of analyses and decrease response time.

Disclosure of Interest: None Declared

Keywords: Diagnostic test, Multiplex immunoassay, Swine pathogens

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-166

The initiative of PRRS area regional control/elimination in Japan (P-JET: PRRS-Japan Elimination Team)

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Introduction: PRRS is one of the most economically significant diseases in the Japanese swine industry. The annual economic loss due to PRRS in Japan was reported as 28 billion JPY ($373 million) (Yamane *et al.*, APVS, 2009).
To initiate PRRS area regional control/elimination in Japan, P-JET (PRRS-Japan Elimination Team) has been founded since July 2011. Objectives of P-JET are the followings:

- To organize a working group that consists of swine practitioners, researchers, and industrial partners who focus on PRRS area regional control/elimination in Japan.
- To establish and provide a network, technical know-how, and educational support for pig producers and veterinarians who are active in their PRRS area regional control/elimination projects in Japan.
- To create and publish a hands-on manual of PRRS control/elimination, which will be tailored to the some of the specifics of the Japanese pig industry. The manual will be available for pig producers and veterinarians in Japan.

Materials and Methods: The main activities of this group are the followings:

- Routine P-JET member meeting (periodically)
- Workshop for each project region and case
- Presentation at industrial and academic seminars
- Publication for industrial and academic journals
- Producing and providing technical/educational materials for pig producers and veterinarians

Results: To date (Dec 2015), a total of 28 P-JET member meetings and 4 P-JET workshops have been completed. A number of seminars and publications have also been made. P-JET herd classification and P-JET biosecurity educational brochure have been established and are being widely used among pig producers and veterinarians in Japan. P-JET biosecurity risk assessment tool (BioAsseT) has been established and its usage is already started. P-JET sampling/testing manual is completed and will be published soon.
Conclusion: This is the first initiative of PRRS area regional control/elimination in the history of Japanese swine industry. P-JET has supported PRRS area regional control/elimination projects as well as each farm case. Currently, 10 Japanese projects in 8 prefectures have been started, including approximately 50,000 sows and 300 sites: Farrow-to-grow (20%) and Farrow-to-finish (80%) sites. To ensure and extend them, P-JET activity will be continued. The next steps are the followings:

- To hold workshops that are more technical-oriented, adapted specifically for each project region or case.
- To establish P-JET management recommendation manual.
- To support to complete a mapping in all the project regions.

Disclosure of Interest: None Declared

Keywords: Elimination, PRRS, regional

Viral and Viral Diseases
PRRS

PO-PW1-101

Comparison of pathogenicity of Vietnamese highly pathogenic and Japanese typical PRRS virus in experimentally infected sows in late gestation

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) is characterized by late abortion and early farrowing in sows. In 2006, highly pathogenic PRRS (HP-PRRS) was occurred and posed a great concern to the global swine industry. Highly incidence of reproductive failures induced by HP-PRRS compared to typical type PRRS were reported. However, differences between HP- and typical PRRS in reproductive failure are not clear. The aim of this study was to compare the pathogenicity of HP- and typical PRRS in late pregnant sows.
Materials and Methods: Seven SPF pregnant sows purchased from PRRSV-free farm were used on 90 days of gestation. Sows were divided to two groups. Group 1 (4 sows) and group 2 (3 sows) inoculated intranasally with $1x10^5$ $TCID_{50}$ HP-PRRSV (100186-614 strain; isolated in Vietnam, 2010) and typical PRRSV (YK09 strain isolated form aborted sow in Japan), respectively. All animals were monitored daily for clinical signs. Serum samples were also collected. Sows were euthanized when they aborted or gave birth prematurely, and tissue samples were collected from sows and fetuses/piglets. Viral RNA in serum samples and several organs were measured by quantitative real time RT-PCR. Histopathological examination was performed on collected tissues, and immunohistochemistry was conducted for same sample with anti PRRSV monoclonal antibody.
Results: In group 1, body temperature increased from 2 dpi, and peaked in 8 dpi (over 40 ℃) with mild respiratory distress. All sows aborted from 11 to 17 days post inoculation (dpi). In group 2, sows showed no clinical signs during experimental periods, and farrowed suddenly between 20 to 22 dpi. Histologically, mild to moderate interstitial pneumonia and small necrotic foci in lymphoid organs with viral antigens were observed in both groups of sows. In fetuses, only a few animals showed small necrotic foci in lung and/or mild lymphadenitis in both groups. Small necrotic foci were detected in the tip of second folds of fetal placenta of group 1 fetuses. Viral antigens were also detected in fetal placental lesions of group 1 fetuses. Viral RNA was detected in serum samples from 1 dpi in group 1 sows and 3 dpi, in group 2 sows. Amount of viral RNA in group 1 and 2 shown approximately 10^5 to 10^6 and 10^3 to 10^4 $TCID_{50}$/ml at 5-10 dpi, respectively. In uterus of sows and fetal organs from both groups, viral RNA was also detected.
Conclusion: In this study, fetal placental lesion with viral antigen was only observed in group 1. Although, mechanism underlying the development of this lesion is still unclear, the lesion might be involved in abortion of HP-PRRS.

Disclosure of Interest: None Declared

Keywords: late gestation, Porcine reproductive and respiratory syndrome virus (PRRSV), reproductive disorder

Viral and Viral Diseases
PRRS

PO-PW1-165

ELIMINATION OF PRRS AND IMPROVEMENT OF PRODUCTIVITY IN SLOVENIAN PIG FAMILY FARMS

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Introduction: A significant decrease in pig meat production has been noticed in Slovenia since 2004. Most of pig farms was small-sized with one-site production system. Because of gradual renovations, farmers applied different technologies within units without biosecurity measures implemented. New technologies were not followed, due to lack of investment. Health status has been decreasing rapidly since 2004, when animals from abroad were introduced to the farms without quarantine or any testing. Major health problem became porcine reproductive and respiratory syndrome virus (PRRSV). The aim of our work was to evaluate acceptance of changes proposed and sow productivity on participating farms.
Materials and Methods: The project was carried out on 16 one-site units with 34 to 79 breeding sows per herd from October 2011 to 2014. Farms participated voluntarily and were willing to improve health status and technology. Technological measures with emphasis on pig welfare were proposed to farmers, e.g. introduction of batch management of breeding sows, optimization of working schedule, improvement of housing conditions and stockmanship focusing on enhance of observational skills. Implementation of stricter external and internal biosecurity measures were proposed together with improvement of health status. Serum samples of breeding animals and fatteners were analysed to detect antibodies against PRRSV (n=2876), APP (*Actinobacillus pleuropneumoniae;* n=166), salmonella (n=166), and leptospira (n=80). The proposed measures for PRRS positive farms were: herd closure with passive immunization (n=1) or serum inoculation (active immunization, n=1), herd closure only (n=7), and control of disease (n=2). Common impact of proposed solutions on fertility was analysed. Statistical analysis was developed by SAS 9.4 package.
Results: Three-week production rhythm was adopted on 13 farms. Rooms were constructed within nursery, farrowing, and finishing units on 5 farms. Half of farmers renovated barns, while 3 farmers decided to build new barns. Diet composition, quantity, and particle structure was corrected. Among 16 farms, 5 farms were PRRSV negative from the start. PRRSV was eliminated on 6 farms, 2 farms improved their health status, while 3 farms did not follow recommendation. Litter size was for 0.54 liveborn piglet larger on farms with implemented modifications, also weaning-to-oestrus interval shortened for 3.50 days and weaning-to-conception interval for 7.61 days. Only on farms which accepted changes proposed, positive trend was observed for fertility traits during the whole period.
Conclusion: Health status and productivity were improved on farms which practised strict biosecurity measures and accepted technological changes.

Disclosure of Interest: None Declared

Keywords: health status, pig family farms, productivity

Poster Abstracts

Viral and Viral Diseases
PRRS

PO-PW1-102

Porcine reproductive and respiratory syndrome virus diversity in herds participating or not in a regional control and elimination program in Quebec

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) is a challenging disease to control at the herd level, hence area and regional control and elimination (ARC&E) programs are increasingly popular around the world. The purpose of our study was to describe PRRS virus diversity in herds participating or not in an ARC&E project within the same administrative region.

Materials and Methods: The ARC&E project which included 43 sites was initiated in 2012 and encompassed an area of 300 km^2. Control measures included more stringent biosecurity measures, vaccination of sow herds and piglets from negative sources, and regular testing (2-4 times/yr). The non-ARC&E area (10 800 km^2) had similar production structure and consisted of approximately 800 sites which, however, were not systematically sampled. PRRSv ORF5 sequences were analyzed over a 4-year period (Jan 2012 - Oct 2015).

Results: A total of 178 and 618 sequences were identified from 43 and 270 different sites in the ARC&E and non-ARC&E areas, respectively. The proportion of vaccine-like (VL) strains increased in the ARC&E herds from 34% in 2012 to 42% in 2015, and from 19 to 35% in the non-ARC&E herds. The overall proportion of wild-type (WT) strains was lower in ARC&E (60%) than in non-ARC&E herds (77%). According to the year, the proportion of strains having ≥97.5% pairwise genetic similarity was 1.2 to 3.7 times higher in ARC&E than in non-ARC&E areas, varying between 10-23% and 5-10%, respectively. For WT strains only, these proportions were 7-20% for ARC&E and 4-10% for non-ARC&E areas, and except for 2012, proportions were 1.5 to 3.2 times higher in ARC&E than in non-ARC&E areas. The number of new strain introductions into a herd (≤92% genetic similarity with herd sequencing history in LEMP database) was similar over the 4-year period in ARC&E (average 7/yr) and, in non-ARC&E herds averaged 27/yr for 2013-2015. This latter number is likely underestimated since sampling was not as thorough as in ARC&E area, and herds without a sequencing history in years 2008 to 2015 were not considered.

Conclusion: The purposes of ARC&E programs are to decrease diversity of circulating strains to stabilize the herds regionally and to decrease the number of PRRS outbreaks. Although the number of new introductions was similar over the years, PRRSv diversity seems to have decreased globally and this was mainly due to vaccination, with high similarity of VL strains, and to a lesser extent to circulating WT strains. Caution should be taken when interpreting results since systematic sampling may overestimate PRRSv genetic similarity in ARC&E herds, and a lower level of submissions in non-ARC&E herds may underestimate the number of new strain introductions.

Disclosure of Interest: None Declared

Keywords: PRRS control, PRRSv gentic diversity

Viral and Viral Diseases
PRRS

PO-PW1-111

Recombinant PRRSV Expressing Luciferase Genes Provide a New Indication of Viral Propagation in Both Permissive and Target Cells

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Introduction: PRRSV has a condensed single-stranded positive-sense RNA genome consists of several overlapping regions. There are at least ten ORFs in PRRSV genome. But only two regions: ORF1b/ORF2a, ORF4/ORF5 without overlap between the adjacent ORFs in type 2 PRRSV genome. The important cis-acting element that function in discontinuous transcription process is TRS. In this study, based on the reverse genetic system of type 2 strain pHuN4-F112, firefly luciferase or renilla luciferase genes were inserted between ORF1b and ORF2. An extra TRS6 was embedded behind the foreign luciferase genes in order to drive ORF2 expression, while make authentic TRS2 regulate exogenous genes transcription. The resultant recombinant plasmids pA-Fluc and pA-Rluc were constructed and rescued successfully in MARC-145 cells.
Materials and Methods: SOE PCR was performed for firefly and renilla luciferase genes insertion into pHuN4-F112. It had advantage that no exogenous restriction endonucleases sites importation. The in vitro transcripts of the mutant clones were transfected into MARC-145 cells to rescue viable virion. Indirect fluorescence assay (IFA) was conducted for detecting trasnslation of PRRSV N protein for mutant and parental viruses. Viral characteristics identification, such as multi-step growth curve, plaque morphology were used for comparison for recombinant and parental viruses. RT-PCR and sequences determination of exogenous genes insertion sites were used for viral genetic stability evaluation. Luciferase activity assay was used for assess vA-Fluc and vA-Rluc infection in MARC-145 cells and PAMs
Results: The recombinant pA-Fluc and pA-Rluc were constructed via reverse genetic manipulations. The recombinant vA-Fluc and vA-Rluc were rescued and could maintain genetically stable in at least 10th cell passage. The progeny viruses vA-Fluc and vA-Rluc showed indistinguishable phenotypically characteristics with the parental virus vHuN4-F112. Multi-step growth curve showed that the peak titer and the propagation tendency were similar, but in the early phase of infection, before 8 hours post infection, the viral titer of vA-Fluc and vA-Rluc were lower than vHuN4-F112. Different time points of mutant virus infected cells were lysis for firefly luciferase and renilla luciferase activity identification to assess the foreign gene expression level. The results showed that the two kinds of luciferase activity variation were identical with its multi-step growth curve in MARC-145 cells and PAMs, which could be used for indicating viral propagation in PRRSV cell cultures.
Conclusion: The recombinant vA-Fluc and vA-Rluc would become new indication tools for PRRSV propagation in both passage cells and target cells.

Disclosure of Interest: None Declared

Keywords: firefly luciferase; renilla luciferase; viral propagation

Viral and Viral Diseases
PRRS

PO-PW1-188

Effect of Aivlosin® and partial depopulation against abortion and post-weaning respiratory symptom caused by PRRSV in a farrow-to-finish Japanese farm

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) has a major economic impact on pig production around the world. In Japan, PRRS virus (PRRSV) still causes huge financial damages, estimated at 28.3 billion Japanese Yen (US$ 370 million) per year. We expect Aivlosin® efficacy and activity *in vivo* against PRRS, this study in a commercial farms is intended to support this assumption.
Materials and Methods: The farm is farrow-to-finish system with 700 sows. As an intervention plan for PRRS, PRRS vaccine is used twice a year in all sows. However, the mortality increased in a weaning unit from 2.2% to over 4%. 40 Sows showed abortion from October to December 2015, max 15% per month even when the abortion ratio was around 1% before. Also fever and anorexia were observed in other pregnant pigs.
Histopathological examination of tissues from pigs with clinical symptoms indicated the possibility of PRRSV infection. Immuno-histochemical staining, histo-pathological examination, PCR test of serum and lung lesion were carried out and the diagnosis was confirmed for PRRS.
Aivlosin® premix was added in the feed, at 100 ppm in the weaning unit and for the sows in lactation and pregnancy period. In addition, partial depopulation of weaning pigs was done from November to December 2015. During this period, runt pigs were completely culled.
Abortion cases and mortality of weaning piglets were measured before and after Aivlosin® administration.
Results: The numbers for abortions were 4.2% in October, 15.1% in November, but decreasing after using Aivlosin® to 4.8% in December. Mortality of weaning piglets was temporarily increased over 4%, but clinical symptoms in the weaning unit improved after administering Aivlosin® in feed.
Conclusion: It has been reported that tylvalosin may inhibit the replication of the PRRS virus, *in vitro*. We confirmed clinical improvement after use of Aivlosin. Immune stabilization of sows is an important measure against PRRSV infection. The use of Aivlosin premix accelerated in this particular case the clinical improvement in the breeding herd. In combination with partial depopulation of the weaning unit, it has been effective to improve the clinical symptoms of the pigs after weaning.

Aivlosin® contains tylvalosin as active ingredient, and is a registerd trademark of ECO Animal Health Ltd., London, United Kingdom.

Disclosure of Interest: None Declared

Keywords: ANTIMICROBIAL, PRRS, TYLVALOSIN

Viral and Viral Diseases
SIV

PO-PT2-030

Elimination of Influenza A Virus in multiple breed to wean herds

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Introduction: Influenza A virus (IAV) is an important infectious agent because of its impact on production and zoonotic potential. Vaccination effectiveness to control the disease is debatable in part due to the constant genetic change of this RNA virus. The goal of this case report is to summarize the protocol and results of herd closure, autogenous whole-herd vaccination and nursery depopulation to eliminate IAV from three 5.000-sows herds.
Materials and Methods: The entire sow herd and gilt developer unit (GDU) beyond the nursery phase were vaccinated twice, three weeks apart, using an autogenous product containing the hemagglutinin (H) antigen(s) isolated from the herd. Gilts received two vaccinations when moved from the on-site nursery to the GDU. Following the initial whole-herd vaccination replacement gilts were weaned to an off-site location until the on-site nursery was depopulated, washed and disinfected. During the time of off-site weaning McRebel management in the farrowing rooms was implemented to reduce transmission. Thirty nasal swabs from each of the two oldest rooms in farrowing were collected 6 and 8 weeks following the initial whole-herd vaccination, and tested in pools of 5 by IAV-PCR to evaluate shedding. Approximately 10 weeks after the initial vaccination, replacement gilts were weaned into the on-site nursery. At this time, the off-site facility was closed to new entries and PCR on oral fluid (OF) samples was used to monitor virus circulation. Seven weeks after closing the off-site facility two mass vaccinations three weeks apart were given with the same autogenous product. At the time of the second vaccination in the off-site facility a third whole-herd vaccination was given at the sow farm to all females. Two weeks later the off-site replacement gilts were brought back into the sow farm. Monitor of clinical signs and PCR testing were performed on the negative gilts in the on-site nursery.
Results: IAV-PCR results at 6 and 8 weeks post whole-herd vaccination were negative in all herds. The off-site weaned gilts tested PCR positive on OF for several weeks but after the mass vaccination IAV RNA was no longer detected. No clinical signs suggestive of influenza or PCR-positive samples have been detected in the herd.
Conclusion: The use of whole-herd autogenous vaccination along with herd closure and partial depopulation is an effective method to eliminate circulating IAV in a sow herd. In two farms the vaccine contained a single antigen and in the most recent farm two different subtypes were included. Although results are encouraging, additional replicates with different strains and farm conditions are necessary to evaluate the repeatability and feasibility of the protocol.

Disclosure of Interest: None Declared

Keywords: Elimination, Influenza A Virus

Poster Abstracts

Viral and Viral Diseases
SIV

PO-PT2-159

Epidemiological evaluation of Swine Influenza in The Netherlands using MSD-AH's ResPig® and IDT's typing

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Introduction: During the last years, H1N1 Influenza dynamics in Dutch pig farms have changed with more clinical problems in sows and nursery pigs due to the reporting of so called endemic influenza. Influenza infections can be responsible for multiple respiratory problem episodes in one nursery batch. Influenza epidemiology based on virus type prevalence and sow seroprofiles was studied over a 12 month period in The Netherlands using ResPig® serology, PCR and virus typing results from IDT
Materials and Methods: On an every six month basis, MSD-AH`s ResPig® provides the opportunity to investigate the influenza status via cross-sectional blood sampling (5 samples/group) of gilts, sows, weaners and nursery pigs, and saliva testing (via PCR) of two groups of just weaned piglets and oldest nursery pig. Vaccination history is also reported. Sows seroprofiles (Idexx Serotype A ELISA) are screened as N (all samples negative), N/P (positive and negative samples) and P (all samples positive). A nursery is called influenza virus positive if one or more saliva samples were PCR positive. Virus prevalence was also calculated. Typing of virus isolated from nasal swabs (IDT) is performed at Friedrich Loeffler Institute (Riems, Germany), using RT-qPCR and Sanger sequencing where possible. The prevalence of the different subtypes was calculated based on the positive nasal swabs.
Results: A total of 120 ResPig® investigations from 12-2014 to 11-2015 were included. The 12 months were divided in 4 quarters: 1= 12/2014-2/2015, 2= 3/2015-5/2015, 3= 6/2015-8/2015, 4= 9/2015-11/2015.The sow seroprofile (%) for each quarter was: N (1:0, 2:0. 3:0, 4:0) N/P (1:11, 2:30, 3:28, 4:19) P (1:89, 2:70, 3:72, 4:81). The Influenza virus prevalence by quarter in the nursery was: 1: 8%, 2: 18%, 3: 24%, 4: 48%.
Subtype prevalence: 217 nasal swabs from weaned piglets were investigated in the period from April to November 2015, of which 61 (28.1%) were positive. The subtypes found were: 33 SIV with no further subtyping possible, 9 H1huN1, 4 H1avN1, 6 H3N2, 6 HxN1 and 3 HxN2. Of the isolates that could be typed further, none were (partly) of pandemic 2009 origin.
Conclusion: In The Netherlands there was a strong increase of influenza virus prevalence in the nurseries in 2015. All sow farms (vaccinating and non-vaccinating) had seropositive sows indicating that there is a high infection risk. The predominant subtype is H1N1.
Further study on specific farms needs to be done to assess the possible role of influenza in clinical problems in sows and piglets, particularly because Influenza is often associated with endemic problems in sows and nurseries in other countries.

Disclosure of Interest: None Declared

Keywords: H1N!, Influenza, typing

Viral and Viral Diseases
SIV

PO-PT2-053

An update of influenza A virus surveillance of swine from the University of Minnesota Veterinary Diagnostic Laboratory

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Introduction: In the 21st century, our understanding of the global diversity and evolution of influenza A viruses in swine (IAV-S) has improved considerably. A more complete picture of the genetic diversity of IAV-S circulating globally has been enhanced by increasing surveillance for IAV-S many pig-producing countries. These increases in surveillance have advanced our understanding of how IAV-S diversity evolves in swine. Described herein is the most recent summary of influenza A virus surveillance testing performed at the Univeristy of Minnesota Veterinary Diagnostic Laboratory (UMVDL). The UMVDL is a fully accredited laboratory that routinely receives and tests porcine samples from North and South America.
Materials and Methods: Respiratory tract samples and oral fluids submitted to the UMVDL were tested for IAV-S matrix gene using a real-time RT-PCR, and virus isolation in MDCK cells of any IAV-S PCR positive samples. HA and NA subtyping of IAV-S RT-PCR positive samples was completed. HA gene sequences were either obtained from virus isolates or directly from the originally submitted material.
Results: Between November 1, 2014 and November 30, 2015, the UMVDL performed IAV-S rRT-PCR Matrix Gene tests on 21,467 samples from pigs in 30 US States, 4 Canadian Provinces, 2 Mexican States, and 4 South American countries. April 2015, July 2015, and October 2015 were the three months with the highest numbers of samples tested. Samples were positive for IAV-S each month, with April and May having the highest the of IAV-S PCR positive results. H1N1, H1N2, and H3N2 viruses were found each month in approximately equal proportions. Only rarely were H3N1 viruses detected. HA gene sequencing of 483 virus isolates revealed the expected genetic diversity, with 6 H1 swine-origin clades and 1 H3 swine-origin clade of influenza A viruses identified. Human-seasonal H1 and human-seasonal H3 clades of influenza A viruses were also identified in the viruses isolated from swine, albeit rarely.
Conclusion: IAV-S are diverse. Human-to-swine transmission, spatial migration via swine movements, and genomic reassortment are the key evolutionary mechanisms that generate this viral diversity, per a 2015 PLOS-Current manuscript by Martha Nelson, Marie Culhane, et. al.. Therefore, additional antigenic characterization and whole-genome sequencing is greatly needed to understand the diversity and independent evolution of IAV in swine.

Disclosure of Interest: None Declared

Keywords: influenza, surveillance

Viral and Viral Diseases
SIV

PO-PT2-074

Field evaluation of RT-LAMP assay for the detection of influenza A viruses from different animal species

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Introduction: Rapid and accurate diagnostic methods for influenza A viruses (IAV) infection are needed for surveillance, outbreak management, and early infection control of emerging influenza viruses. The LAMP characteristics of rapidity, simplicity, high sensitivity, and specificity make it a powerful tool for disease diagnostics that has been widely applied to human, animal, and plant pathogen detection. Recently, we developed a RT-LAMP assay for swine IAV (SIV) detection that was very useful for detecting major subtypes of SIVs (H1N1, H1N2, and H3N2). However, the usefulness of the RT-LAMP assay for detection of other animal IAVs was not evaluated, although primers were designed for detection of all animal IAV subtypes. Therefore, we also evaluated the performance of the RT-LAMP assay with respect to animal IAVs, including major subtypes of avian, canine, and equine influenza viruses, as well as field samples in the present study.
Materials and Methods: The RT-LAMP reaction was carried out using IAV matrix gene-specific six primers. RT-LAMP reaction mixture containing 1ul Bst DNA polymerase (8 U/ul, New England Biolabs, Ipswitch, MA, USA), 5ul template, reverse-transcriptase (10 U/ul, Invitrogen, Carlsbad, CA), 2.5ul dNTPs (10 mM), 8ul Betaine (250 mM), 1ul MgSO4 (150 mM), 1ul HNB (3mM, Lemongreen, Shanghai, China) and 1ul of each primer. The sensitivity of the RT-LAMP assay was determined and compared with RT-PCR and real-time PCR using the same template at identical concentrations. To evaluate the usefulness of the RT-LAMP assay for detection of all IAV subtypes, a total of 21 animal IAVs, including AIV, SIV, EIV, CIV, human IAV, and a human influenza B virus, were tested.
Results: The RT-LAMP detection limit was 10^{-2}-, 10^{-3}-, 10^{-1}-, and 10^{-1}-$TCID_{50}$ for each tested AIV, SIV, EIV, and CIV, respectively, which was 10-fold higher than those observed from RT-PCR results and the same as those observed for RRT-PCR, as predicted by previous reports. RT-LAMP clinical performance was evaluated by using 589 field samples collected from different animal species. The RT-LAMP assay detected eight samples as IAV-positive, which was consistent with RRT-PCR and virus isolation results, and subtypes of IAV-positive cases were confirmed as H1, H3, H5, H6, or H9 subtype.
Conclusion: In this study, we evaluated our previously reported RT-LAMP assay for the detection of animal IAVs, including major subtypes of avian, swine, canine, and equine influenza viruses. The RT-LAMP assay evaluated in this study is applicable for rapid, user-friendly, and reliable screening of IAV in animal populations and is expected to be an alternative method to RT-PCR or RRT-PCR, even in under-equipped laboratories.

Disclosure of Interest: None Declared

Keywords: None

Viral and Viral Diseases
SIV

PO-PT2-158

Seroprevalence of swine influenza virus on swine farms in the Philippines

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Introduction: Swine influenza virus (SIV) infection in pigs is dependent upon the infecting virus strain, age and immune status of the animals and concomitant pathogens. In the Philippines, SIV infection can either be quick onset and rapid recovery or can be less obvious and demonstrate traditional clinical signs of infection. Not all farms are vaccinating against SIV because the specific strains are not identified and prevalence of the different SIV subtypes circulating in swine herds is unknown. Therefore, the purpose of this study was to determine the seroprevalence of swine influenza viruses in farm herds that do not vaccinate.
Materials and Methods: A cross-sectional collection of sera was conducted between mid-2014 and mid-2015 in 13 farms (no SIV vaccination) located in major swine producing provinces with respiratory diseases. Samples from each farm range from 20 to 40 sera (average of 36) taken from breeder sows, weanling, growers and finishers. A total of 475 pig sera were tested for influenza A antibody using Influenza A Virus Antibody Test kit (IDEXX Influenza A Test Kit) to measure exposure of a herd to Influenza A. The sera were also tested using hemaglutination-inhibition (HI) technique with European Isolates which included: SWI type H1N1-Best; SWI type H1N2-Gent and SWI type H3N2-Flanders 1/98. An inhibition of haemagglutination at dilution >= 4 (log2) are regarded to be positive.
Results: Numbers and percentages of seropositive animals according to age group, aggregated across the 13 farms, were evaluated. Seroprevalence of influenza A viruses is 65%. Seropositivity in each subpopulation is variable with the highest % in growers and sows (80%). The seroprevalence of SIV subtypes as determined with the HI test was as follows: H1N1 132 sera (28%), H1N2 9 sera (2%) and H3N2 52 sera (11%). Seropositivity in each subpopulation was variable among H1 and H3 subtypes. The sow herd had the highest seroprevalence (59%) for H1N1 subtypes positive sera.
The level of antibody seropositivity between the Influenza A antibody test and HI test was numerically different across all sera and among age groups. This difference could be attributed to the European strains of SIV used in the HI test.
Conclusion: This study demonstrated that natural infection rate of SIV is highly prevalent in Philippine swine herds and that it occurs in all stages of production. The three active influenza subtypes (H1N1, H1N2 and H3N2) are present. Further studies on strain prevalence should be conducted to provide clearer understanding on the prevalence of European SIV subtypes and American SIV subtypes in Philippine pigs

Disclosure of Interest: None Declared

Keywords: HI Test, Influenza A Antibody test, SIV seroprevalence

Poster Abstracts

Viral and Viral Diseases
SIV

PO-PT2-016

Circulation of the novel influenza virus, proposed as influenza D virus, in Italian pig farms.

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Introduction: Recent studies have identified a novel influenza virus circulating in swine and cattle. The virus, distantly related to human influenza C virus, has been provisionally designated as influenza D virus (IDV) and a new genus of the *Orthomyxoviridae* family was proposed. This novel virus was identified for the first time in pigs with influenza-like illness, but subsequent serologic and virological studies conducted in USA and in France have suggested cattle as a possible reservoir.
Materials and Methods: To investigate the circulation of IDVs among pigs in Italy, we performed biomolecular and virological tests on clinical samples collected from herds affected by respiratory distress located in Po Valley, the area in Italy with the highest density of swine and cattle farms. During a 5-month period spanning August 2015-December 2015, 530 samples of nasal swabs (n. 219), lungs (n. 211) and oral fluids (n. 100) were collected from 273 pig farms.
We screened clinical specimens by reverse transcription quantitative PCR. All positive samples were confirmed by partial polymerase basic 1 gene sequencing and submitted to viral isolation in CACO-2, HRT18 and ST cell cultures. RNA was isolated from IDV positive clinical samples or positive supernatants and full-genome amplification and sequencing were performed. Phylogenetic analysis of viral gene segments was carried out.
Results: Screening of samples by biomolecular test (PCR) found that 17 samples (3.2%) from 4 different farms (1.4%) were positive for IDV. Among the 17 positive specimens 14 were nasal swabs, 2 were oral fluids and only one was a lung tissue .
IDV was isolated on CACO-2, HRT18 and ST cell cultures from 3 nasal swabs collected in two positive farms. Genetic analysis highlighted that Italian IDVs are very closely related belonging to the D/swine/Oklahoma/1334/2011 cluster.
Conclusion: Our findings show that one cluster of IDV circulate among swine affected by respiratory disease in Italy. In all the considered clinical cases other viral and / or bacterial pathogens were detected in IDV positive specimens but in samples from one farm where mild symptoms were observed. These findings confirm the presence of IDV in Italian herds. Nevertheless it is difficult to outline the pathogenic role of IDV in swine respiratory distress complex. Considering the higher percentage of positive nasal swabs it seems that upper respiratory tract is a preferred location. Further studies are ongoing to better understand epidemiology, virology, pathobiology and zoonotic aspects of IDV, especially concerning the evidence that Koch's postulates are fulfilled for this agent.

Disclosure of Interest: None Declared

Keywords: influenza D virus, swine

Viral and Viral Diseases
SIV

PO-PT2-051

COUNTERACTION OF SEVERE LOSSES CAUSED BY A SWINE INFLUENZA VIRUS REASSORTANT H1N2 (AVIAN ORIGIN HEMAGGLUTININ) AFFECTING REPLACEMENT STOCK IN SPAIN

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Introduction: Swine influenza is characterized by respiratory clinical signs, fever, and reproductive disorders. The most prevalent subtypes of swine influenza viruses (SIV) are H1N1, H1N2 and H3N2. New viral variants may emerge due to reassortment. The aim of this clinical case presentation was to describe losses in replacement stock caused by SIV and their control by means of vaccination.

Materials and Methods: In April 2014, nursery and finishing pigs of a two-site SPF farm of 950 sows in Spain showed respiratory problems. Blood samples from sows/piglets were negative to porcine reproductive and respiratory syndrome virus (PRRSV) and oral fluids were positive to Influenza A virus and negative to PRRSV. In May 2014, a batch of 87 PRRSV and *Mycoplasma hyopneumoniae* negative gilts was introduced in the farm. One week later, gilts showed cough and fever; 11 died and 2 more were culled. Subtype H1N1 was detected by RT-PCR in oral fluids. It was decided to vaccinate gilts at the entrance of quarantine with Respiporc FLU3 (IDT Biologika). This measure was not completely efficient for the following 5 batches of gilts (n=453). Gilts still showed respiratory signs one week after entering quarantine, 7 died, 11 were sent to slaughter and one was euthanized. Blood was taken before entering quarantine and 13 nasal swabs at the time of clinical signs appearance. These samples were tested by SIV RT-PCR including subtyping.

Results: Gilts were seronegative to SIV at the entrance, but 12/13 samples were RT-PCR positive at the time of clinical signs. Subtyping of 5 samples gave H1N2, with an avian hemagglutinin origin. The veterinarian decided to change the first SIV vaccination schedule, with a first Respiporc FLU3 shot 3 weeks before entering the quarantine and the second one at the day after entrance. In June 2015, the first batch of gilts was introduced to quarantine. Vaccination against SIV highly reduced respiratory signs, mortality rate and use of drugs. Only 1 out of 90 gilts was culled due to a rectal prolapse. Similar data were obtained in the following replacement batches.
Conclusion: An apparent multiple SIV strain co-infection (H1N1 and a relatively novel H1N2) in a naïve herd caused high mortality and increased number of culled gilts. Vaccination against SIV with sufficient time to develop a proper immune response before infection controlled clinical signs associated with the infection.

Disclosure of Interest: None Declared

Keywords: swine influenza virus (SIV); gilts; vaccination

Viral and Viral Diseases
SIV

PO-PT2-212

Genetic evolution of recently emerged novel human-like swine H3 influenza A viruses (IAV) in United States swine

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Introduction: Influenza A virus (IAV) is a major cause of respiratory disease in swine. IAV transmission from humans to swine is a major contributor to swine IAV diversity. In 2012, a novel H3N2 with an HA (hu-H3) and NA derived from human seasonal H3N2 was detected in United States (U.S.) swine. The hu-H3 continued to be detected with evidence of reassortment with endemic swine IAV. Swine isolates from 2012 and 2014 with hu-H3 were shown to be fully virulent and transmissible in swine. We conducted a phylogenetic analysis of sixty-eight hu-H3 detected between 2012-15 in the USDA surveillance system and from the Iowa State University Veterinary Diagnostic Laboratory.
Materials and Methods: Representative whole genome sequences of North American swine and human IAV were compiled with our data. Sequences were aligned and we inferred maximum-likelihood trees in RAxML. Each gene was assigned to an evolutionary lineage (human, classical swine, TRIG, or 2009 pandemic H1N1 (H1pdm09)). A time-scaled Bayesian approach was implemented for the HA gene in BEAST. The deduced HA1 amino acid sequences were used to identify amino acid differences between human and swine viruses.
Results: An increasing frequency of detection was demonstrated with 3 hu-H3 detected in 2012, 8 in 2013, 17 in 2014, and 40 in 2015. Viruses with the hu-H3 showed at least three reassortment episodes: early viruses contained a human-origin N2; second generation viruses contained a classical swine N1; and third generation viruses contained a swine 2002 N2, with early viruses containing an H1pdm09 backbone and later viruses containing TRIG lineage and H1pdm09 internal genes. The first detection in the USDA swine IAV surveillance system was Nov 2012, but time to the most recent common human H3 ancestor was between Nov 2010 and Feb 2011. These viruses likely went undetected for up to two years, potentially the result of reduced viral replication or transmission in swine during this time. The swine hu-H3 gene differed at up to 25 amino acid positions from closely related human H3, including 8 mutations in recognized antigenic sites.
Conclusion: Surveillance data demonstrates this novel IAV lineage is established in the U.S. swine population. It spread from detection in a single state in 2012 to 7 states in 2015, with an increasing percentage of isolates in the national surveillance system (3% in 2014; 5% in 2015). These viruses are a significant threat to the industry, as antigenic distance from the swine H3 circulating since 1998 indicates current vaccines will likely have limited efficacy. The risk of transmission of swine IAV with hu-H3 and H1N1pdm09 internal genes back to humans should be considered.

Disclosure of Interest: None Declared

Keywords: Human-like, Influenza, Swine H3

Viral and Viral Diseases
SIV

PO-PT2-007

GENETIC DIVERSITY OF SWINE ORIGIN INFLUENZA A VIRUS IN CHILE AND THE LATIN AMERICAN PERSPECTIVE

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Introduction: Influenza A virus (IAV) is an important pathogen in swine production. In Chile, IAV has been reported in clinical cases since 2009. However, only few farms have been studied and there is limited information regarding genetic diversity. Recently several Latin American (LA) countries have reported and published IAV gene sequences. The objective of this study was to analyze the genetic diversity of IAV in Chile and to compare the genetic diversity in LA countries.
Materials and Methods: Twenty seven Chilean intensive swine farms were sampled between 2013 and 2015. These farms are representative of modern swine pig production. Nasal swabs (NS) and oral fluids (OF) samples were obtained in each visit and tested by Real-time RT-PCR (qPCR) and virus isolation (VI). Selected positive samples were sequenced using full genome sequencing by Illumina.
Bayesian Evolutionary Analysis Sampling Trees approach was used to reconstruct the phylogenies. Reference sequences published in GenBank were included in the analysis, including available sequences from LA countries such as Mexico, Brazil, Argentina and others. Genetic distances were calculated using Kimura 2-parameter model.
Results: A total of 1500 samples were collected during 50 visits (83% NS and 17% OF). From the total number of samples tested, 347 (23%) were positive. 20% of NS and 34% of OF were positive. Finally we generated a total of 50 new IAV whole genomes. Phylogenetic analysis identified the circulation of 6 IAV genotypes in Chile, which included pH1N1-like, human like H1N2, human like H3N2, and reassortant viruses from them. Human like H1N2 (45%) and pH1N1 (45%) were the most frequently identified strains.
In the LA context, the most remarkable findings were (1) the pH1N1 virus was found in all countries included; (2) classical H1 IAVs were observed only in Mexico and Colombia; (3) in Chile, Argentina and Brazil human like H1 IAVs were found and they are not closely related with Delta Cluster viruses; and (4) Human like H3 viruses were observed in Chile, Argentina, Brazil and Mexico.
Conclusion: Human like H1N2 virus is frequently detected in Chilean pig farms. This virus is genetically different from H1 clusters seen in North America and not related to any other reported IAV. The phylogenetic tree constructed suggests independent human-to-swine introductions of the pH1N1 strain in Chile as well as the rest of LA. Results suggest that the human like IAVs are common in Argentina, Brazil and Chile, which are different from the rest of IAV reported in pigs. Results from this study can be considered to improve prevention and update the vaccines used LA.
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Disclosure of Interest: None Declared

Keywords: influenza virus, Latin America, SIV

Poster Abstracts

Viral and Viral Diseases
SIV

PO-PCO1-008

Pathogenesis and transmission of highly pathogenic avian influenza H5Nx in swine

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Introduction: Influenza A viruses (IAV) periodically transmit between pigs, people, and birds. If two IAV strains infect the same host, genes can reassort to generate progeny virus with potential to be more infectious or avoid immunity. Pigs pose a risk for such reassortment. Highly pathogenic avian influenza (HPAI) viruses are a global health concern due to the high human case fatality rate observed with specific H5 lineages, such as the Euro-African lineage H5N1 that emerged in the Middle East in 2005. HPAI of Eurasian H5 lineage were recently detected in wild birds and backyard and commercial poultry in North America, with an H5N2 causing outbreaks in the Midwest. *In vivo* studies were performed with North American HPAI H5's, the Euro-African H5N1, and Euro-African H5N1 and H1N1pdm09 reassortants to assess the risk of these HPAI H5 in pigs.
Materials and Methods: Wildtype viruses from Clade 2.3.4.4 (H5N8, H5N1, and H5N2), Clade 2.2 (H5N1), H1N1pdm09, and Clade 2.2 (H5N1) and H1N1pdm09 reassortant viruses were inoculated into primary challenged pigs. Contact pigs were comingled on 2 days post-infection (dpi). Serum was tested by ELISA and hemagglutination inhibition (HI) assays. Nasal swabs were taken to evaluate viral shedding. Lungs were removed at necropsy and bronchoalveolar lavage fluids (BALF) were collected. Lungs were examined for pneumonia and for histopathologic evaluation. Viral RNA was extracted and quantitative TaqMan real-time PCR assays were performed. Virus isolation was performed on RT-PCR positive samples.
Results: There was limited replication of avian HPAI of Clade 2.3.4.4 in primary pigs and no evidence for spread to contact pigs. However, Clade 2.2 lineage H5N1 showed some transmission in pigs that increased by swapping the H5 and N1 surface genes onto the H1N1pdm09 internal gene backbone. HPAI was detected by the RT-PCR screening test used by veterinary diagnostic labs in the U.S. for swine and may also be detected by H5 HI assays. However, not all primary pigs seroconverted at the time points tested, so further work is required to determine the reliability of HI to assess pig exposure to H5.
Conclusion: HPAI showed a restricted ability to infect pigs, but the potential for HPAI viruses to incorporate genes from H1N1pdm09 to become more infectious to pigs remains. However, no reassortant viruses with HPAI H5N2 genes were reported from the 2015 North American poultry outbreak and most avian H5 viruses fail to replicate or transmit in pigs. Infection and sustained transmission seems to be unlikely, although the risk is not zero. Continued surveillance for circulating or novel strains in swine and poultry is critical for early detection.

Disclosure of Interest: None Declared

Keywords: None

Viral and Viral Diseases
SIV

PO-PT2-094

International ring trials for adoption and validation of real-time RT-PCR protocols for sub-typing European swine influenza viruses

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Introduction: Swine influenza is a major economically important disease in pig populations across Europe. Four sub-types ($H1_{av}N1$, H1N1pdm09, $H1_{hu}N2$ and H3N2) of swine influenza A viruses (swIAVs) plus various reassortant combinations have been isolated in pigs. Diagnostic tests capable of rapidly identifying the sub-types of the currently-circulating strains within the pig population are crucial. Traditional serological methods for HA and NA sub-typing are laborious, costly, time-consuming and interpretation of results suffers from non-specific cross-reactions of test antigens. Conventional assays are unable to detect novel viruses or distinguish the emergence of new reassortants. Real-time RT-PCR (rtRT-PCR) formats can improve the sensitivity and speed of swIAV sub-typing and overcome shortcomings with conventional formats. Protocols will retain the high sensitivity of the rRT-PCR and facilitate sub-typing of swIAV rRT-PCR-positive samples directly from clinical material which might be negative by virus isolation or conventional sequencing methods.
Materials and Methods: Representative swIAV isolates were tested in an initial ring trial for comparison of the molecular and conventional sub-typing protocols employed across seven laboratories. The best-performing primers/probe sets and amplification protocols, already validated and used routinely in France and Germany, were run in simplex rtRT-PCR assays specific for detection of H1 of the different genetic lineages ($H1_{av}$ and $H1_{hu}$), for H3, and in duplex assays for detection of N1 ($N1_{av}$) and N2. A second ring trial was undertaken and the protocols used by other partners to test clinical material in addition to amplified virus.
Results: These harmonized protocols enabled all partners to achieve successful and specific identification of HA and NA genes of swIAVs from enzootic European lineages. The assays provided fast results, enabled a semi-quantitative assessment of virus loads with differentiation between the pandemic and avian-like H1N1 virus strains and identification of HA/NA reassortant viruses. Data from the testing of clinical material from the United Kingdom (UK) and partner laboratories will be presented. Promising results have been achieved following the identification of H1N2 and $H1_{av}N1_{av}$ swIAV strains in previously virus isolation-negative, untyped influenza A-positive (matrix gene) swabs and tissues (n=37, 43% full HA&NA, 35% partial HA or NA, 22% no result) submitted for testing from the UK swine influenza surveillance program.
Conclusion: The harmonized protocols will facilitate the sub-typing of previously uncharacterized swIAV strains directly from clinical material by PCR without having to depend on virus isolation and nucleotide sequencing.

Disclosure of Interest: None Declared

Keywords: molecular sub-typing, swine influenza virus, swine influenza virus sub-typing

Viral and Viral Diseases
SIV

PO-PT2-020

The persistence of influenza A viruses in swine breeding herds

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Introduction: Influenza A viruses (IAVs) are a main cause of respiratory disease in pigs and endemic in North American swine, however the mechanisms that allow IAVs to persist in pig herds are not clearly understood. The objective of this longitudinal study was to characterize the genetic diversity of IAV in pig breeding herds using next generation sequencing technologies.
Materials and Methods: Five pig breeding herds were selected for this study. In each herd 90 nasal swabs were collected on a monthly basis during a year from new gilts (replacement animals on site for less than 30 days, n=30), gilts (replacement animals on site for more than 30 days, n=30), and piglets (n=30). All samples were tested for IAV by RT-PCR and positive samples were used for IAV isolation using MDCK cells. Positive IAV isolates were used for deep genome sequencing using next generation sequencing technologies (NGS). IAVs were classified based on the HA and NA combination and compared to IAV circulating in North America between 2003 and 2014.
Results: All farms and pig subpopulations tested positive for IAV at least once and 123 IAVs were successfully sequenced. Viruses from the same monophyletic group were found for 183 days within the same herd. Seven different genetic clusters were identified during the study period and represented three different IAV subtypes (H1N1, H1N2 and H3N2). However, several reassortment events were found.
Conclusion: In conclusion our results demonstrate the complexity and plasticity of IAV genetic diversity in pig breeding herds and highlights the advantage of NGS technologies to understand virus diversity and evolution.

Disclosure of Interest: None Declared

Keywords: Complete genome sequencing, Next-generation sequencing , Swine influenza A virus epidemiology

Viral and Viral Diseases
SIV

PO-PT2-052

The antigenic diversity of influenza A viruses during infection of weaned pigs.

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Introduction: Influenza A viruses (IAVs) are endemic in North American swine and cause a respiratory disease. However the mechanisms that allow IAVs to persist in pigs after weaning are not clearly understood. The objective of this study was to characterize the antigenic diversity of IAV during two contiguous epidemic waves of infection in pigs after weaning.
Materials and Methods: One hundred and thirty two pigs were randomly selected at arrival to a wean-to-finish farm. Nasal swabs were collected from all pigs on a weekly basis for 15 weeks and tested for IAV by RT-PCR. Ninety-two positive samples were selected for deep genome sequencing using next generation sequencing (NGS) technologies. Hemagglutinin (HA) and Neuraminidase (NA) sequences were compared at the nucleotide level to other IAVs circulating in the USA. Amino acid sequences were used to construct median-joining networks and polymorphic amino acid sites were estimated.
Results: Two IAV epidemic waves were identified within 10 weeks after pigs were weaned and two different IAV subtypes (H1N1 and H3N2) were recovered. However, deep genome sequencing allowed us to differentiate three different viral groups (VG). While VG1 contained H1 gamma viruses, VG2 and VG3 contained H1 beta and H3 cluster IV viruses respectively. Although VG1, VG2, and VG3 viruses were identified at several sampling events, VG1 dominated the first epidemic wave of infection and VG3 dominated the second one. Furthermore, the number of polymorphic amino acid sites among the antigenic proteins of viruses from VG1 was higher than the number of polymorphic amino acid sites found in VG2, VG3.
Conclusion: Our results demonstrate that several IAV genotypes can co-circulate in pigs after weaning and that the proportion of genotypes over time can be dynamic.

Disclosure of Interest: None Declared

Keywords: Influenza A virus complete genome sequencing, Influenza A virus epidemiology, Swine influenza antigenic diversity

Poster Abstracts

Viral and Viral Diseases
SIV

PO-PCO1-006

Effect of maternally-derived antibodies on influenza infection and antibody response in pigs after weaning

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Introduction: Maternally-derived antibodies (MDA) against influenza A virus (IAV) have been associated with improved clinical presentation in pigs and in certain instances, decrease on transmission. In this study, we assessed the effect of MDA on IAV infection and antibody response in pigs after weaning under field conditions.
Materials and Methods: A cohort of 132 pigs was identified at weaning and sampled weekly by collecting nasal swabs during 15 weeks. Blood samples were also collected at weaning and every 4 weeks. Sera were assayed for antibodies by ELISA as well as hemagglutination inhibition (HI) test including field and vaccine strains (H1N1 Gamma, H1N1 Delta 2, H1N2 Delta 1, and H3N2 Cluster IV). Nasal swabs were tested by RT-PCR targeting the matrix gene. Cut-off point for ELISA S/N values was 0.6 and for HI titers it was 40.
The association between ELISA S/N values and time-to-infection was tested using the Spearman correlation test. The association between HI titers and time-to-infection was tested using the Kruskal-Wallis test. The influence of MDA levels on antibody response after infection was evaluated using the Wilcoxon test.
Results: Two waves of influenza infection were identified. The first wave was dominated by an H1N1 Gamma 1 virus and peaked at week 2 post weaning. Ninety percent of pigs tested IAV RT-PCR positive by then. Second wave of infection was dominated by an H3N2 Cluster IV A virus, peaked at week 7 and 72% of pigs tested RT-PCR positive by week 8. In addition, 56% of pigs were positive by ELISA at weaning but only 30% remained seropositive at week 4. Almost all pigs were seropositive again at weeks 8 and 12. Finally, 17% and 0% of pigs at weaning had HI titers ≥ 40 against field strains, H1N1 and H3N2 respectively. Moreover, between 33% and 68% of pigs at weaning had HI titers ≥ 40 against various vaccine strains.
There was no association between ELISA status at weaning (pos/neg) and time-to-infection after weaning. Same results were obtained when HI status (pos/neg) for the 6 strains tested was associated with time-to-infection after weaning. The antibody response according to serostatus at weaning, either by ELISA or HI, showed that pigs that were seropositive at weaning had lower levels of antibodies at 8 weeks post weaning when compared to the ones that were seronegative.
Conclusion: Our study indicated co-circulation of distinct IAV strains in a wean-to-finish population. Also, it suggested that the rapid spread of IAV after weaning was related to the low levels of MDA. We showed some level of MDAs interference in the antibody response after infection and provided some insights into the level, type and variability of IAV antibodies after weaning.

Disclosure of Interest: None Declared

Keywords: Influenza A Virus, maternally-derived antibodies, weaned pigs

Viral and Viral Diseases
SIV

PO-PCO1-007

An emerging H1N2 sub-cluster within the alpha H1 cluster of influenza A viruses of swine in Canada

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Introduction: Over the last 15 years, genetically and antigenically distinct groups of influenza A viruses in swine (IAV-S) have emerged. The 2009 pandemic H1N1 virus and variant H3N2 viruses of swine-origin underscore the threat that IAV-S pose to public health. Consequently, there is greater need for increased IAV-S surveillance globally. This is particularly important in regions where there is very little historical sequence data available. In this study, IAV-S surveillance was conducted to examine H1N1 and H1N2 sequences from Western Canada.
Materials and Methods: Diagnostic samples of lung tissue and nasal swabs were collected from pigs in Alberta (AB), Saskatchewan (SK) and Manitoba (MB). Samples were screened for IAV-S by CFIA or USDA certified Matrix PCR tests. Positive samples were subtyped and virus isolation and sequencing of the hemagglutinin (HA) gene was attempted on 443 viruses. Whole-genome sequencing was performed on 169 of these viruses. Phylogenetic analyses were conducted in MEGA 6 using Neighbor-Joining and Maximum Composite Likelihood methods with bootstrapping.
Results: Phylogenetic analysis of the HA genes revealed that the Western Canadian H1 viruses (2012-2015) were within the pH1N1 and H1α clusters. Within the H1α cluster there were two distinct sub-clusters. These included the H1N1α viruses known to previously circulate in this region and a new cluster of H1N2 alpha viruses first detected in MB in the fall of 2013. Most of the H1N2α viruses contained the same 2 amino acid (AA) deletion (amino acid positions 129 and 130 in the H1 numbering system), which is similar to the 1AA human-like deletion observed in the H1 delta-cluster viruses. This H1N2 strain containing a unique deletion at amino acid positions 129 and 130 continues to be the dominant IAV-S detected in Western Canada 2015. Whole genome analysis revealed the same phylogenetic pattern for the other 7 genes of these H1N2α viruses. While 17/36 virus sequences are from the 6 farms in one MB system, 12 are from unrelated farms in MB, 1 unrelated farm in SK and 6 farms in the United States. Additionally, one strain from AB and two strains from MB are within the H1N2 alpha sub-cluster, but do not contain this deletion.
Conclusion: The results highlight the importance of IAV-S surveillance within overlooked regions, and is thus crucial for understanding the ecology and spatial dissemination of swine diseases. In order to develop effective control strategies for emerging influenza strains, we need to know what types of viruses are circulating. We also need to know the genetic relationships of these viruses to each other, as well as their relationship to viruses in other regions.

Disclosure of Interest: None Declared

Keywords: emerging porcine viruses, Influenza A Virus, Molecular epidemiology

Viral and Viral Diseases
SIV

PO-PT2-019

Vaccine efficacy of live-attenuated, whole inactivated and alphavirus vectored vaccines against antigenically distinct H3N2 swine influenza A viruses

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Introduction: Influenza A virus (IAV) is an important pathogen in swine, and the main intervention strategy is vaccination to induce neutralizing antibodies against the hemagglutinin (HA). Three major antigenic clusters, cyan, red, and green, were identified among H3N2 viruses circulating in pigs in the U.S. and were associated with amino acid changes in 6 key sites in the HA protein. In this study we compared the efficacy of different vaccine platforms including adjuvanted whole inactivated virus (WIV), live-attenuated influenza virus (LAIV), and an alphavirus vectored vaccine against challenge strains that were antigenically distinct.
Materials and Methods: Three animal experiments were carried out: experiment 1 used cyan antigenic virus WIV and LAIV against a heterologous red antigenic virus; experiment 2 used alphavirus vectored monovalent and bivalent vaccines expressing the HA of green and/or red antigenic viruses against homologous and heterologous challenge; and experiment 3 used green antigenic virus WIV and LAIV against homologous green and heterologous red challenge.
Pigs were challenged with each assigned virus, and nasal swabs were collected at 0, 1, 3, and 5 days post-infection (DPI) from pigs. At 5 DPI, lung and trachea lesions were evaluated and bronchoalveolar lavage fluid collected. Virus isolation, virus titer, hemagglutination inhibition (HI) assays, lung lesion scoring and mucosal antibody quantitation were performed.
Results: In these studies, reduced cross-reactivity in HI assays was observed when tested against heterologous antigens, validating the significance of the cyan, red and green antigenic clusters. The levels of cross-protection against antigenically distinct challenge viruses varied across the vaccine platforms with LAIV as the most effective, the alphavirus vectored vaccine as intermediate, and the WIV providing the least amount of cross-protection. Enhanced lung lesions were observed when pigs were vaccinated with the green WIV and challenged with the red antigenic virus.
Conclusion: The main strategy used to prevent or reduce morbidity of IAV in swine is to employ commercially available and farm-specific autogenous vaccines. These studies will help to define the importance of the 6 antigenic sites to vaccine efficacy and indicate the number of H3N2 viruses required to be included in multivalent vaccines to cover the breadth of antigenic variants of H3 swine IAV co-circulating in the U.S. Amino acids at these 6 antigenic positions in the HA were associated with antigenic cross-reactivity of swine H3 IAV in the U.S. and attention to these sites could facilitate the rapid detection of antigenically drifted viruses.

Disclosure of Interest: None Declared

Keywords: H3N2, Live-attenuated vaccine, Subunit vaccine

Viral and Viral Diseases
SIV

PO-PT2-021

Monitoring and characterization of swine influenza virus (swIAV) in Europe since 2015

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Introduction: Influenza A virus infections causing economic losses are widely spread among swine populations worldwide. Swine can be infected by avian as well as by human influenza viruses. In Europe, over the last decade, three stable lineages of reassortant viruses between avian and human viruses have formed. The human pandemic H1N1/2009 virus has become a fourth player in this field and is currently disturbing the balance of the previously established European porcine influenza virus lineages. The emergence of the most recent human pandemic influenza virus (H1N1/2009) from reassortant porcine influenza viruses underlines the importance of swine populations as carriers of influenza lineages with zoonotic and even pandemic potential. In view of the OneHealth concept a closer surveillance of these populations therefore seemed a logical consequence of the most recent human influenza pandemic. However, surprisingly few countries actually embarked on sustained, governmentally driven and publicly controlled monitoring programs. It is therefore conceivable that, after closure of the EU-financed ESNIP3 program, a passive surveillance program for swIAV in selected European countries has been initiated on basis of funding by a veterinary vaccine producer.
Materials and Methods: This project targets nasal swab samples collected from pig farms with clinically apparent respiratory problems in European countries. Samples are screened by real time RT-PCR (RT-qPCR) for presence of influenza A viruses. Positive samples are subjected to molecular subtyping, virus isolation, antigenic and phylogenetic characterization.
Results: About a quarter of the pigs with clinically apparent respiratory problems was found to be infected with influenza A virus. Three porcine H1 and one H3 lineages as well as various reassortant between them were detected. Prevalences of the different lineages are geographically restricted.
Conclusion: A high incidence of influenza virus infections representing all four lineages and various reassortants between them was detected in a season-independent manner. Incursions of new lineages and/or reassortants were documented for several European countries. Surveillance will continue into 2017 and comprise up to 6.000 samples.

Disclosure of Interest: None Declared

Keywords: Influenza A, surveillance , swine

Poster Abstracts

Viral and Viral Diseases
SIV

PO-PT2-022

Reassortment of avian H1N1 and swine H3N2 influenza viruses in pigs*

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Introduction: A genetic reassortment of influenza viruses of different hosts is an important mechanism for overcome the species barrier. Therefore the aim of this study was to define the percentage of reassortants and their gene constellation after co-infection of pigs with 2 influenza virus strains of different subtype and origin.
Materials and Methods: Six pigs were intranasally inoculated with A/Swine/Gent/172/08 (H3N2) and A/Duck/Italy/1447/05 (H1N1) viruses to study the likelihood of genetic reassortment in the pig. At 2 DPI, 6 naïve pigs were introduced.
Swabbing and body temperature check were performed daily. At 4 DPI/DPC 3 infected and contact exposed pigs were euthanized and samples from the respiratory tract were tested by qRT-PCR (M gene). Blood samples for HI test and IPMA were collected at 14, 21 and 28 DPI/DPC.
All positive in the qRT-PCR samples were used for plaque purification and virus isolation. The genetic characterization of isolated viruses was executed with use of different molecular methods (RT-PCR, PCR-RFLP, sequencing).
Results: Virus shedding was detected from 1 to 6 DPI/DPC in the infected and contact exposed pigs. From all samples positive in the qRT-PCR in total, 202 and 26 isolates from tissue and swab samples were obtained, respectively. In both cases most of them (for tissue about 65%; for swabs more than 90%) were isolate from contact exposed pigs.
A differentiation between avian and swine origin of all genes of obtained isolates was done. All viruses from tissue were H3N2, whereas from swabs 18 isolates were H3N2 and 8 isolates (3.5%) were H1N1. All H1N1 viruses were isolate from contact exposed pigs. Among the H1N1 isolates 3 strains (1.3%) were reassortants with one gene of swine origin (NP or M). All collected sera were seronegative for H1N1 and seropositive for H3N2 in HI test, whereas in IPMA all of them were seropositive for both H1N1 and H3N2 virus.
Conclusion: Based on the results we can state that for the reassortment to occur co-infection of pig with two or more influenza virus and their efficient replication must take place. However the reassortment is a very complex process and different factors such as location of virus replication, its virulence and titer, a host susceptibility e.t.c, may influence on its occurrence. Under experimental conditions we manage to achieve the reassortation between H1N1 and H3N2 viruses of different origin, however the percentage of reassortants suggests low genetic compatibility of viruses used in the study.
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Disclosure of Interest: None Declared

Keywords: influenza virus, pig, reassortment

Viral and Viral Diseases
SIV

PO-PT2-057

Effect of vaccination against pdmH1N1(2009) influenza virus on reproductive performance

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Introduction: Swine influenza is a worldwide appearing pathogen which causes important losses in swine production. The disease is characterized by sudden onset, coughing, dyspnea and fever. Apart from these clinical signs, swine influenza can also result (directly or indirectly) in abortion, fetal death or other reproductive disorders. Since 2009, the subtype pdmH1N1(2009) has been isolated with an increasing prevalence in pigs worldwide, likely indicating that this subtype has become established in the swine population. The aim of the current study was to assess the reproductive performance parameters before and after a vaccination against pdmH1N1(2009) influenza virus in pdmH1N1(2009) affected herds. Therefore, the reproductive performance parameters of 42 farms were assessed six months before and six months after vaccination with an inactivated panH1N1 vaccine.
Materials and Methods: Reproductive performance parameters of vaccinated sows against pdmH1N1(2009) influenza virus were determined using an application observation sheet in 42 commercial pig farms that were either serologically or virologically pdmH1N1(2009) positive. The performance data were compared based on sows organizer data of the respective farm for the time points six months before and six months after vaccination to determine the site-specific level of reproductive performance before and after vaccination; with special emphasis on return to estrus and the abortion rate of sows. The collection of data was distributed throughout the year to exclude seasonal influences. Statistically all data were analyzed by Testimate Version 6.5.
Results: Valid data regarding the return to estrus could be collected for 42 farms. Before vaccination the return to estrus was between 3.4% to 33.3% (Ø 13.2% ± 7.4) and after the implementation of vaccination between 2.3% to 24.1% (Ø 9.6% ± 5.0). Therefore, a mean improvement of 3.6% could be detected six months after vaccination ($p = 0.0095$). Valid data to abortion rate exist in 23 application observation sheets of the 42 of investigated farms, which show a decline in the abortion rate from 3.1% ± 2.9 (min: 0.2%; max: 10.0%) before use of the inactivated pdmH1N1(2009) vaccine to 1.6% ± 1.9 (min: 0.0%; max: 6.4%) post vaccination ($p = 0.0266$).
Conclusion: According to the results the abortion rate and the return to estrus could be significantly diminished six months after vaccination in comparison to the performance data six months before the establishment of vaccination. Owing to the vaccination with a inactivated pdmH1N1(2009) vaccine reproduction failures in farms with detection of this virus could be significantly reduced.

Disclosure of Interest: None Declared

Keywords: reproductive parameters, Swine Influenza, Vaccination

Viral and Viral Diseases
SIV

PO-PT2-220

Longitudinal assessment of swine influenza infection in commercial farrow-to-finish pig farms in São Paulo state, Brazil.

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Introduction: Swine Influenza is an infectious viral disease with high morbidity and related to several productive losses such as growth retardation, abortion in pregnant sows and predisposition of infected animals to other respiratory infections. Swine Influenza Virus (SIV) seems to be more widespread in pigs than previously thought. This research focused on assessing and comparing the seroprevalence of SIV in different age groups in commercial farrow-to-finish pig farms in São Paulo state, Brazil.
Materials and Methods: During the year of 2015, five commercial farrow-to-finish pig farms in the northeastern region of São Paulo state were sampled. Each age groups (sows, suckling piglets, nursery and finishing pigs) present in the farm had approximately 30 animals randomly chosen for sample collection. In total, 606 swine serum samples were obtained. The detection of specific anti-influenza antibodies was done using a commercial ELISA kit based in a H1N1 strain presenting cross-reaction with antibodies against of H3N2 and H1N2 (CIVTEST-Suis, *Laboratorios* Hipra SA) which has a sensibility of 98% and 100% of specificity. The test was performed according to the manufacturer's instructions.
Results: The seroprevalence values obtained in each herds were: Farm 1 - 45% (92.31% of suckling piglets, 6.67% of nursery pigs and 70.37% of sows); Farm 2 – 20% (30.00% of suckling piglets and 45.45% of sows); Farm 3 – 5% (20.00% of sows); Farm 4 – 38% (44.83% of suckling piglets, 25.00% of nursery pigs, 16.67% of finishing pigs and 60.00% of sows) and at last Farm 5 – 4% (13.33% of suckling piglets and 3.03% of sows). As can be seen, positive animals were detected in almost every age groups, probably related to the high morbidity feature of the disease allied with the densely populated pens and barns, which enables the virus to quickly spread among the swine herds. In all farms, the sow group had the high seroprevalence values since these animals stay for longer periods in the farms and consequently have more odds of becoming infected along her life than the other animals. The seroprevalence in suckling piglets suggest the presence of colostral-derived antibodies.
Conclusion: Swine influenza was detected in all tested farms, suggesting that this disease is widespread in pig farms of São Paulo state. The highest risk group is the sows and, there seems to be no serious problems with SIV in nursery and finishing pigs in farrow-to-finish pig farms which could be associated with Porcine Respiratory Disease Complex. This study had financial support provided by Primeiros Projetos - Edital 12/2015-PROPe / UNESP.

Disclosure of Interest: None Declared

Keywords: ELISA, seroprevalence, SIV

Viral and Viral Diseases
SIV

PO-PT2-242

Comparison between the occurrence of swine influenza in intensive pig farming and non-technified pig herds

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Introduction: Pigs can be infected with avian, swine and human influenza A viruses, and for that reason, swine has been classically proposed to be the mixing vessel where reassortant influenza strains can arise. It is of common belief that non-technified pig herds are more susceptible to infectious diseases due to the lack of biosecurity measures in the animal rearing. The present research focused on assessing and comparing the occurrence of swine influenza in intensive pig farming and non-technified pig herds.
Materials and Methods: A set of 361 swine serum samples of 56 different herds from non-technified farms and 612 samples from five commercial farrow-to-finish pig farms were obtained in order to accomplish this research. All of these farms were located in the northeastern region of the State of São Paulo, Brazil. At each of those intensive pigs farming, 30 serum samples were collected from each age group: sows, suckling piglets, nursery and finishing pigs and, in the non-technified herds, the age group were adults (breeding pigs) and young (growing pigs). The detection of specific anti-influenza antibodies was done using a commercial ELISA kit based in a H1N1 strain presenting cross-reaction with antibodies against of H3N2 and H1N2 (CIVTEST-Suis, *Laboratorios* Hipra SA) which has a sensibility of 98% and 100% of specificity. The test was performed according to the manufacturer's instructions.
Results: There were no positive samples (0.00%) out of the 361 from non-tehcnified herds. Regarding to intensive pig farming, 23.36% (143/612) of samples were detected anti-influenza antibodies at the ELISA test. The results opposites the idea that non-technified herds are more prone to have diseases due to the lack of the adoption of biosecurity measures in animal rearing. It seems that characteristics of the industrial pig production system could favor the spread of some infectious agents. When it comes to airborne diseases such as swine influenza, dense population, the lack of air circulation in pens and barns and at industrial pig site enables the swine influenza virus to spread faster in the herd when compared to non-technified herds, in which the animals are reared in low populated pens and have fewer contact with humans.
Conclusion: The swine influenza was not detected in non-technified pig herds e was demonstrated SIV infection in intensive pig farming, suggesting that maintenance of the swine influenza strains keeps in industrial pig production. This study had financial support provided by Primeiros Projetos - Edital 12/2015-PROPe / UNESP.

Disclosure of Interest: None Declared

Keywords: different pig production systems, ELISA, SIV

Poster Abstracts

Viral and Viral Diseases
SIV

PO-PT2-201

Evidence of Swine Influenza Virus infection in Tayassuidae from commercial rearing in Brazil

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Introduction: Family *Tayassuidae* in the suborder *Suina* include two species of peccaries that are found in Brazil: the whitelipped peccary (*Tayassu pecari*) and the collared peccary (*Pecari tajacu*). This animals is medium-sized hoofed mammal and share many characteristics with domestic and wild swine (*Sus scrofa*), including potential reservoir of infectious diseases due some common pathogens. In Brazil, there is commercial rearing of peccary for meat production. Swine Influenza Virus (SIV) is widespread in pig population, especially in intensive pig farming. Despite of capacity to cause disease in pigs, should be considered its zoonotic potential. There are absences of data about swine influenza infection in *Tayassuidae* in Brazil. This research aimed to detect anti-swine influenza antibodies in *Tayassu pecari* and *Pecari tajacu* from commercial rearing in Brazil.
Materials and Methods: From two commercial rearing of peccaries were obtained 105 serum samples which were 50 of *Pecari tajacu* from Goiás state and 55 of *Tayassu pecari* from Minas Gerais state. The farms were specialized in the production of peccaries and have permission from environmental authorities. Collecting of blood samples occurred at slaughter of animals. For detection of specific anti-influenza antibodies was done using a commercial ELISA kit based in a H1N1 strain presenting cross-reaction with antibodies against of H3N2 and H1N2 (CIVTEST-Suis, *Laboratorios* Hipra SA) which has a sensibility of 98% and 100% of specificity. The test was performed according to the manufacturer's instructions.
Results: The seroprevalence values of SIV infections obtained in peccaries herds was 10.47%, however, were not found anti-SIV antibodies in *Tayassu pecari*. Regarding to *Pecari tajacu* the seroprevalence was 22%. In the epidemiological investigation was checked that the herds were reared in semi intensive system, no mixing with other species of animals, balanced feed supplied and had contact with human due the management. Source of infection is questionable because it is not known if it came from own animals in wildlife or cross infection with human. This report is the first demonstration of swine influenza infection in peccaries from commercial rearing in Brazil.
Conclusion: Swine influenza was detected in *Pecari tajacu* suggesting that this disease might be present in wild species. However, influenza surveillance on peccaries is essential to monitor viruses circulating. We don't know if novel influenza viruses can be generated in peccaries by reassortment like pigs. Thus, the role of SIV infections in peccaries still remains unknown and more investigations are needed.

Disclosure of Interest: None Declared

Keywords: ELISA, peccaries, SIV

Viral and Viral Diseases
SIV

PO-PT2-075

Using oral fluids samples for indirect influenza A virus surveillance in farmed UK pigs

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Introduction: Influenza A virus (IAV) is economically important in pig production and has broad public health implications. Because of public health concerns, some geographic areas have IAV monitoring in swine initiated. In Europe, active IAV surveillance includes demonstration IAV RNA in nasal swabs or oral fluids and/or demonstration of antibodies in serum (SER) samples; however, collecting appropriate numbers of individual pig samples can be costly and labor-intensive. The objective of this study was to compare the sensitivity and specificity of oral fluid (OF) and SER samples in detecting anti-IAV antibodies.
Materials and Methods: Twenty-seven commercial pig herds located in the UK were included in this study. Paired SER and OF samples were collected from all farms; 70.4% (19/27) farms were sampled on one occasion only and the remaining 29.6% (8/27) of the farms were visited at the time of weaning until around 12 weeks of age in approximately two week intervals. While OFs were collected at each time point, SER samples were collected during the last visit only. A commercial nucleoprotein (NP)-based blocking ELISA was used to test 244 OF and1004 SER samples from 123 pens each containing 20-450 pigs. At least two OF samples were selected per farm for detection of IAV matrix gene RNA by rRT-PCR (n = 92).
Results: There was a strong positive correlation ($r = 0.7584$; $P < 0.0001$) between the average pen SER and the OF sample to negative (S/N) ratio. The number of pens classified as IAV seropositive with at least one positive SER or OF sample, was higher for SER than for OF (66.9% vs. 37.1%, $P < 0.001$). The overall agreement between qualitative ELISA data between paired samples was moderate ($\kappa = 0.43$) and increased with the increase of within-pen positive SER samples ($P < 0.05$). The highest agreement occurred for pens in which more than 60% of the SER samples were IAV antibody positive. While anti-IAV antibodies were detected in 62.9% (17/27) of the investigated farms, IAV RNA was only detected in 7.4% (2/27) of the farms.
Conclusion: Serological assays provide a number of benefits compared to molecular detection of IAV, the most important one being the ability to detect IAV exposure after active viral replication has ceased. Under the study conditions, SER samples provided a higher detection rate of IAV antibodies when compared to OFs. Collecting more than one OF sample in pens with more than 25 pigs should be considered in future to further elucidate the suitability of this sample type for IAV surveillance in herds with large pen sizes.

Disclosure of Interest: None Declared

Keywords: Influenza A Antibody test, Oral fluids

Viral and Viral Diseases
SIV

PO-PT2-076

Production impact of influenza A(H1N1)pdm09 virus infection on fattening pigs in Norway

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Introduction: Influenza A(H1N1)pdm09 virus infection although observed in a subclinical form in Norwegian pigs, can lower the pig's growth performance by reducing feed efficiency in terms of a poorer feed conversion ratio. Infected pigs consume more feed and require protracted production time to reach market weight. Our stochastic models were constructed to simulate the summed negative effects of the infection at the batch level of 150 fattening pigs growing from 33 to 100 kg.
Materials and Methods: Observational longitudinal growth performance data from 728 control pigs and 193 infected pigs with known viral shedding time points were analyzed using mixed linear regression models to give estimates of the marginal effects of infection. Gaussian curves describing the variability of the estimates at the individual pig level formed the fundamental inputs to our stochastic models. Other inputs of variability and uncertainty were 1) batch transmission points, 2) pig infection points to reflect the disease transmission dynamics of the virus, and 3) final prevalence of infected pigs in the batch. Monte Carlo random sampling gave 5,000 estimates on the outputs of the marginal effects for each pig. These results were summed up to provide estimates for a batch size of 150 pigs. This figure was adjusted by our final prevalence distribution function, which was also derived from the longitudinal study with 12 cohorts of infected pigs.
Results: For a 150-fattening-pig herd randomly selected from the population, the marginal effects of the infection were 1) 835 kg (fifth percentile) to 1,350 kg (95th percentile) increased feed intake and 2) 194 (fifth percentile) to 334 (95th percentile) pig days in excess of expected figures for an uninfected batch. A batch infected during growth phase 3 (81 to 100 kg BW) gave the worst results since the longitudinal study showed that a pig infected during growth phase 3 required more feed and a greater protracted production time compared to younger infected pigs. Sensitivity analysis shows that final prevalence had the greatest impact on the conditional mean and variation of the marginal effects of infections. Batch transmission point was the next most influential factor.
Conclusion: Impact of a lower feed efficiency caused by influenza A(H1N1)pdm09 virus infection in fattening herds can be minimized by lowering the final animal prevalence and preventing older fattening pigs from being infected. This will give the greatest benefit in saving feed cost and reducing delay in getting the pigs to the market. **doi:10.2527/jas.2015-9251**

Disclosure of Interest: None Declared

Keywords: feed efficiency, Influenza A (H1N1)pdm09 virus, stochastic models

Viral and Viral Diseases
SIV

PO-PT2-190

FREQUENCY ANALYSIS OF H1N1 AND H3N2 ANTIBODIES IN SAMPLES FROM PIG FARMS IN MEXICO (2012-2015)

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Introduction: The objective was to investigate the frequency of antibodies against H1N1 and H3N2 subtypes of Influenza A virus (IAV) in pig farms in Mexico from May 2012 to June 2015, using the hemagglutination inhibition test (HI).
Materials and Methods: A total of 3,444 serum samples were collected from 96 pig farms located in 13 states: Chiapas (61), Coahuila (35), Guanajuato (198), Hidalgo (169), Jalisco (933), State of Mexico (35), Michoacan (74), Morelos (35), Puebla (450), Queretaro (168), Sonora (975), Veracruz (256) and Yucatan (55). Three IH tests were developed from the following Influenza A virus isolates: H1N1 classic A/swine/NewJersey/11/76 (H1N1c), H3N2 reference A/swine/Minnesota/9088-2/98 (H3N2r) and the Mexican isolate H3N2 A/swine/Mexico/Mex51/2010 (H3N2 iso51), since previous studies have shown that this strain has an antigenic behavior different to the H3N2 reference. Samples were considered positive >1:80 dilutions.
Results: At country level, 58.3% of the samples were positive to H1N1c, 34.7% to H3N2r and 33.6% to H3N2 iso51. Percentage positive to more than one test were: 25.7% to H1N1c and H3N2r, 30.6% to H1N1c and H3N2 iso51, and 18.9% to H3N2r and H3N2 iso51. There were 18.0% of samples that tested positive to the three tests. Approximately, half of the farms sampled for this study had been vaccinated against Influenza, and all of them tested positive. At state level there was at least one farm testing positive to each one with the exception of Morelos; all samples from that state where sourced from one single farm not vaccinating against IAV. At farm level, 88.5% had positives to H1N1c, 84.3% to H3N2r and 74.0% to H3N2 iso51. By production category the serum samples breakdown is as follows: 2,905 growers-finishers, 339 breeding sows and 200 pigs of unknown category. 57.6% of growers-finishers were positive to H1N1c, 35.8% to H3N2r and 32.8% to H3N2 iso51. 58.4%, 25.7% and 31.6% of the samples from breeding sows were positive to H1N1c, H3N2r and to H3N2 iso 51, respectively.
Conclusion: Influenza A viruses are widespread in Mexican pig populations. Both H1N1 and H3N2 subtypes are present in commercial farms not vaccinating against those, suggesting they are common. The study shows evidence of the presence of the reference isolates, including the Mexican specific H3N2 (iso 51). The diagnostic results of an HI test for influenza virus will depend on the test. It is important to conduct various studies for the prevention and control of this disease, considering the likelihood that new subtypes may be affecting pigs. Currently there are new diagnostic tools that will help to know in detail the current situation of the disease in Mexico.

Disclosure of Interest: None Declared

Keywords: Influenza, Mexico, Serology

Poster Abstracts

Viral and Viral Diseases
SIV

PO-PT2-119

Identification of Influenza A Virus in pigs from serological studies in Southern and Southeastern Brazil in 2014 and 2015

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Introduction: Influenza A viruses (IAV) were first isolated from pigs in Brazil in 1974, but later studies reported a low seroprevalence of H1N1 and H3N2 subtypes in swine before the 2009 influenza pandemic. After the pandemic in humans, outbreaks in swine were reported in several countries, including Brazil. Serological data indicate that the pandemic subtype, H1N1pdm09, has become endemic in Brazilian herds, with a high prevalence of positive herds. Recently, molecular characterization studies have shown an increased seroprevalence of IAV of human origin (H1N2 and H3N2) in pigs.
Materials and Methods: Serum samples from 50 commercial farms with no history of vaccination against IAV were collected in 2014 and 2015, for a serological survey of IAV subtypes circulation in Southern and Southeastern Brazil (states of Minas Gerais, São Paulo, Paraná, Santa Catarina and Rio Grande do Sul). Samples were collected randomly from 15 animals per production stage in four categories: breeding females (sows and gilts), nursery piglets, growing and finishing pigs, totaling 60 samples per farm. A total of 3,000 samples were tested by the haemagglutination inhibition (HI) assay using three Brazilian IAV isolates from pigs with respiratory clinical signs, collected in 2014, and characterized molecularly as H1N1pdm09, H3N2 human-like and H1N1 human-like (H1N1hu). Samples with HI titers equal or greater than 40 were considered positive.
Results: The percentage of total pigs and farms presenting antibodies against H1N1pdm09, H3N2 and H1N1hu were 36%, 17%, 4% and 94%, 68%, 48%, respectively. Co-infection with H1N1pdm09 and H3N2 was observed in 36% of farms. The H1N1pdm09 subtype was the most prevalent in animals and farms of the five states studied, ranging from 85 to 100% and 22 to 61%, respectively. The prevalence of H3N2 on farms was higher in Paraná (89%) and lowest in Minas Gerais (50%). The H1N1hu was the least prevalent in farms (25 to 67%) and animals (1 to 8%), suggesting low transmission of this isolate among pigs. The category breeding females had the highest percentage of seropositive animals for all tested viruses and the growing and finishing pig category had the highest percentage of susceptible animals.
Conclusion: The IAV subtypes analyzed are widespread in pig farms in the South and Southeast regions of Brazil, with H1N1pdm09 being the most prevalent. In most farms tested co-infections were found. The pig category with the highest seroprevalence was breeding females; nursery and growing/finishing pigs had a lower seroprevalence, indicating increased susceptibility to infection by IAV.

Disclosure of Interest: None Declared

Keywords: swine influenza virus (SIV); serological profile; Brazil

Viral and Viral Diseases
SIV

PO-PT2-008

Maternal antibodies do not prevent infection and propagation of swine influenza virus in experimental conditions

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Introduction: Recurrent influenza infections in swine herds are characterized by swine influenza A virus (swIAV) infections occurring at a fixed age in successive batches, when a significant part of the piglets still have swIAV maternally derived antibodies (MDAs). Although passive immunity is known to provide partial protection against infection, its impact on transmission is not fully understood. The present study aimed at estimating the protective impact of MDAs derived from sow vaccination on swIAV transmission parameters.
Materials and Methods: A transmission experiment involving 72 specific pathogen free (SPF) piglets with or without MDAs was carried out. MDA-positive piglets were derived from vaccinated (Gripovac®3) SPF sows. In each group (MDA-positive/MDA-negative), 2 seeder-pigs per room, inoculated intra-tracheally with a H1N1 virus at 35 days of age, were put in contact with 4 direct- and 5 indirect-contact piglets (3 replicates per group, 2 pens per room). Individual virus shedding (RT-PCR) and MDA waning (ELISA test) were monitored from nasal swabs (daily basis) and blood samples, respectively. The duration of passive immunity persistence as well as the duration of shedding period were estimated using parametric survival analysis. Differential swIAV transmission rates depending on piglets' initial serological statuses and contact structure (direct contact with penmates or airborne route) were estimated using maximum likelihood method, allowing the estimation of specific reproduction numbers.
Results: Time to maternal antibody waning was 71.3 [52.8 – 92.1] days on average. The duration of shedding period was 6.1 days [5.9 - 6.4] for both groups. The airborne-related transmission rate was 0.69 [0.33 - 1.18] newly infected piglets per day leading to a rapid transmission to indirect-contact groups, further inducing within-pen transmission. Based on the transmission rate in MDA-positive and MDA-negative piglets and duration of shedding period, the reproduction number estimates were 5.7 [1.2 - 12.8] and 17.1 [8.9 - 29.4] respectively.
Conclusion: The presence of MDAs in piglets reduced but did not prevent early-life swIAV spread. As the virus spreads more slowly, the period with shedding animals is then expected to be longer at the population scale, which could increase swine flu within-herd persistence. Limitation of practices such as cross-fostering and mingling at weaning which enhance virus spread through direct contacts appears thus pivotal to control swIAV within-herd persistence. Likewise, airborne transmission was identified as a key component, highlighting the need to consider infectious aerosols in swIAV between-room spread process.

Disclosure of Interest: None Declared

Keywords: basic reproduction number, maternally derived antibodies, Swine Influenza

Viral and Viral Diseases
SIV

PO-PT2-199

Risk factors for first and recurrent influenza virus shedding in nursery pigs

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Introduction: Swine influenza outbreaks are usually recognized by the sudden appearance of respiratory signs and also by quick recovery of sick animals. However, influenza A virus (IAV) can endemically circulate without causing such typical clinical outbreaks. In addition, the complexity of influenza circulation in large multi-site and multi-source herds has not been well described. The objectives of this study were to describe the dynamics of IAV circulation in multi-source nursery herds and identify risk factors for recurrent infection.
Materials and Methods: The study was conducted at a ~2000-head nursery operated on an all-in/all-out basis. Pigs from 5 different sow-herds, each with a different health status, were mixed in 4 rooms (each with 24 pens). In the first 2 hours of arrival 400 pigs were selected for the initial virological testing. Additionally, 81 and 75 pigs were included for ongoing weekly testing for influenza virus for Study 1 and 2, respectively. Virus isolation and propagation were done in Madin-Darby canine kidney (MDCK) cells. Serology was performed by hemagglutination inhibition (HI) using 8 different influenza viruses. Risk factor analysis for virological positivity and likelihood of recurrent infection was conducted using logistic regression and survival analysis.
Results: In Study 1, at ~30 days post-weaning, 100% of pigs were positive and shedding viruses, with 35 (43.2%) pigs being positive recurrently. A different pattern was observed in Study 2 with 36 (48%) pigs being positive only once and 8 (10.7%) pigs being positive recurrently. Results indicated that IAV can circulate during the nursery phase in a cyclical pattern and the likelihood of recurrent infections was higher for pigs with higher level of heterologous (within-subtype) maternal immunity, but only for the H3N2 strain, which could explain ongoing issues in the nursery. However, the presence of high heterologous immunity is not likely to explain all recurrent infections because pigs with low heterologous infections were also noticed to be recurrently infected. High degree of within-pen clustering was also observed, suggesting that transmission within a pen played an important role.
Conclusion: Prolonged or recurrent IAV infections could be very important when trying to control IAV infection in nursery barns. These findings could be useful in developing control strategies.

Disclosure of Interest: None Declared

Keywords: Influenza A Virus, recurrent infection, Risk factors

Viral and Viral Diseases
SIV

PO-PT2-198

A Field Comparative Study of Vaccination against Influenza A Virus in Argentinean Herds

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Introduction: In Argentina, since the first outbreak of influenza A virus (IAV) in pigs in 2008, respiratory diseases shifted from bacterial or mycoplasma to viral infections, particularly IAV. Field studies of the efficacy of commercial vaccines against IAV have not been reported in Argentina.
The aim of this study was to compare the infection dynamics of IAV in endemic infected herds with and without the use of a commercial IAV vaccine.
Materials and Methods: A cross-sectional study was carried-out in 2 non vaccinated (NVH) and 2 vaccinated (VH) farrow-to-finish herds. In VH, the vaccine FluSure XP® (Zoetis) was applied in breeding stock. Thirty nasal swabs and 30 blood samples were obtained from breeding (**S**) and 21 (**A**), 46 (**B**), 68 (**C**) and 100 (**D**) days-old randomly selected pigs.
Antibodies against IAV were detected by ID Screen Influenza A ELISA kit (Montpellier, France). Nasal samples were pooled and viral RNA was extracted using a QIAampViral RNA Mini kit (Qiagen, Germany). Real-time RT-PCR (*q*RT-PCR) to detect the M gene of IAV was applied. Positive samples were inoculated in MDCK cells, and sequenced. Chi square was applied to compare proportions of seropositive pigs.
Twenty six necropsies were performed and tissues samples were collected for complementary studies.
Results: The percentage of seropositive pigs in NVH showed a higher variability within **S** (range: 40-80) and **A** (0-65) groups than VH. Percentages of seropositive pigs in groups **S**, **A**, **B** and **C** were significantly higher in VH than NVH ($p<0,0001$).
Both NVH and VH had the same detection rate of IAV by qRT-PCR, more than 90% of the pooled samples of **B** and 1/5 pooled samples of **D** group were positive. From NVH, H1N1pdm09 was identified. From VH, sequencing of isolated virus remains in progress.
Lesions associated with IAV were observed in 3 lungs from NVH and one from VH in which 2 pigs had PCV-2 associated lesions.
Conclusion: In Argentinean pigs multiple lineages of the H1N1, H1N2 and H3N2 subtypes are circulating that are distinguishable from similar North American subtypes. Among them, H1N1pdm09 is the most prevalent. The vaccine applied incorporates the above subtypes however, with those strains currently circulating in North America.
PCR results showed that IAV virus was actively circulating on all farms placing susceptible pigs at risk. VH farms had a higher percentage of seropositive pigs, which would suggest increased resistance to clinical disease if the antibodies were protective. However, the overall incidence of IAV associated lung lesions was too low to allow a firm conclusion on disease prevention.

Disclosure of Interest: None Declared

Keywords: endemic infection, Influenza A Virus, Vaccine

Poster Abstracts

Welfare and Nutrition

PO-PC02-010

Antibiotic growth promotor replacement by a synergistic butyrate based product in piglets

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Introduction: Since 2006, Antibiotic growth promotors (AGP) are banned in the EU. Questions are raising in EU as well as in other parts of the world about the effectiveness of alternatives to replace AGPs. Commonly used alternatives in EU are products based on butyric acid. Butyric acid (C4) is a short chain fatty acid (SCFA) with a biological role, consistently present in the intestinal ecosystem, as it is naturally produced by fermentation of polysaccharides by the intestinal microbiota. Butyrate is seen as an AGP alternative as it plays a major role in promoting gut health by promotion of the intestinal barrier, modulation of the immune system, balancing the intestinal flora and improving digestion and absorption of nutrients, leading to enhanced performance. The aim of this trial was to evaluate the effect on performance of a butyrate based product comparing to the use of a traditional AGP in piglets.
Materials and Methods: A trial was carried out during 35 days at the Swine Research Unit of PUCPR, located in Fazenda Rio Grande, Brazil. 48 weaned piglets were divided into two groups of 8 replicates (Colistin (C) and Butyrate (B)) and were housed in 1,92m^2 nursery pens with slatted floor. 2 corn and soybean meal based diets were formulated according to animals' age: prestarter (1 to 14 days of evaluation) and starter feed (15 to 35 days of evaluation). Treatments consisted of a group treated with Colistin (C) at 40 ppm in prestarter and starter feed and a group supplemented with a calcium butyrate based synergistic product (B) (Butifour® NF) at 0,15% in prestarter and 0,075% in starter phase. Body weight (BW) and feed consumption were controlled weekly to provide data on feed conversion ratio (FCR). Additionally, the incidence of diarrhea was also verified daily, and a score was attributed according to VASSALO et al. (1997): 1 – normal or soft feces, 2 – pasty feces and 3 – aqueous feces. Collected data was analyzed using the software Stata® (Statacorp) by a mixed linear model, except of fecal score data. These were analyzed by Dunn test with Bonferroni adjustment. All statements of difference were made considering P≤0,05.
Results: At the end of the evaluation, no significant differences between B and C were observed regarding FCR (1.563 vs 1.565, respectively), body weight (26.745 vs 26.811, respectively) and diarrhea scores (weighted mean of 0.083 vs 0.067, respectively).
Conclusion: In this trial, a synergistic butyrate based supplementation of piglet feed was regarded to be a suitable alternative for the use of AGPs.

Disclosure of Interest: None Declared

Keywords: AGP, butyrate, performance

Welfare and Nutrition

PO-PC02-011

Inclusion of slaughter line lesions in breeding values for better animal welfare

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Introduction: Slaughter line observations can be included in breeding values of breeding pigs to enhance animal welfare. By monitoring lesions/remarks on joints, lungs, hearts and other parts of the carcasses, clear differences between farms and individual pigs can be demonstrated. Lesions like bursitis, pericarditis and pneumonia indicate lower welfare of the animals. Higher incidences of these lesions are associated with lower average daily gain. These lesions are quite heritable (bursitis 15%, pneumonia 6%, pleuritis 12%, pericarditis 19%, joint lesions 17%). So genetic selection can be expected to reduce these lesions in the progeny.
Materials and Methods: Data from a large meat packer in Germany collected between July 2011 and September 2014 from 90024 pigs were used. The data showed the following average incidences of lesions/remarks

Pneumonia 12.2%
Bursitis 12.1%
Joint lesions 4.6 %
Pleuritis 3.1%
Pericarditis 2.4%

Results: There are clear differences of lesion/remarks between farms. Farms with lower welfare considerations have a higher incidence of lesions and lower average daily gain. Male pigs showed a higher incidence of lesions than female pigs. Carcasweight however was not influenced by the lesions. *The boars were split into two classes based on their breeding values for high and low incidence of remarks.* The incidences of different lesions in their progeny were as follows;

	High(10%)	**Low (10%)**
Nr Sires	70	70
Nr Progeny	5904	5712
Bursitis	17	6
Pneumonia	10	8
Pleuritis	2	2
Pericarditis	2	2
Joint lesions	6	4
Total lesions	**37**	**22**

The observations in the progeny also showed a clear relation with several breeding value classes (graph will be shown at congress).
In view if these favorable results, breeding values for bursitis, pneumonia, pleuritis and pericarditis were combined into an index based on their respective economic values for calculation of the welfare index (TWI) for each pig.
Conclusion: These data show that slaughterhouse observations can be used to include in breeding values (TWI). A large database of individual slaughter lesions is needed. So far this was only carried out in Germany.
Boars with a high TWI are expected to have progeny with fewer remarks when slaughtered and monitored.
This has a benefit in welfare for the animals as they suffer less from leg and joint disorders next to pneumonia and pericarditis. The incidence of these lesions is associated with a lower profit. So the TWI inclusion will also lead to a better economic result for producers and contribute to better welfare for the entire pork value chain.

Disclosure of Interest: None Declared

Keywords: Genetic Resistance, Slaughterhouse, welfare

Welfare and Nutrition

PO-PC02-017

Impact of a live yeast strain: Saccharomyces boulardii CNCM I-1079 on intestinal gene expression of piglets at weaning.

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Introduction: *Saccharomyces cerevisiae boulardii* is a probiotic yeast with an established history of use for the prevention and/or treatment of diarrhoea and chronic inflammatory disease in human medicine. More recently, *S. boulardii* has also been used in the pig industry to increase performance and reduce pathogen burden during vulnerable early life stages such as post-farrowing and weaning. Recent advances in DNA sequencing technology are now able to understand the host response to stress and nutritional intervention: it was the objective of this study.
Materials and Methods: In a blinded randomised controlled trial, we investigated the effect of a *S. cerevisiae boulardii (SCB)* strain (CNCM I-1079) on intestinal gene expression in piglets through the weaning transition.Oral supplementation of piglets with SCB (3.3 x 10^9 CFU/day) started at 7 d of age and continued daily until 35 d. At 21 d of age, piglets were randomly selected to be weaned and mixed with non-littermates or to remain suckling with their dam. Control piglets were subject to the same procedure without SCB. Gene expression analysis at 1, 4 and 14 days post-weaning were performed by qRT-PCR focusing on innate immune genes, and Illumina sequencing was performed on colonic tissue.
Results: Gene expression network and pathway analysis - key targets for the understanding of weaning disorders and the mode of action of SCB - highlighted that weaning in non-supplemented piglets affected the expression of more than 1000 genes in the colon. Genes with the highest fold change were associated with proteolytic degradation of connective tissue, inflammatory responses and epithelium defence against bacterial and viral invasion. In contrast, the number of genes affected by weaning in SCB piglets was reduced by 60% compared to control animals, with the highest fold change genes related to metabolism and transport but none to activation of proteolytic damages, suggesting a possible protective role of SCB on gut barrier function at weaning. Evidence of immunoregulation by SCB in the small intestine, towards an anti-inflammatory profile was associated with the upregulation of Toll-like receptors and Interleukin-10 mRNA levels.
Conclusion: The transcriptomic approach allowed us to generate hypothesis on the mode of action of *S. cerevisiae boulardii* in the gut during the weaning transition. Our results suggest that weaning is a period of intense stress that profoundly affects gene expression in the gut and that *SCB* may alleviate the consequences of weaning by promoting regulatory immune responses and maintaining gut barrier integrity. Mechanism underlying these modes of action are under investigation.

Disclosure of Interest: None Declared

Keywords: live yeast, gene expression, weaning

Welfare and Nutrition

PO-PC02-019

TRAUMATIC NEUROMA DEVELOPMENT IN TAIL DOCKED PIGLETS IS NOT ASSOCIATED WITH LONG-TERM CHANGES IN SPINAL NOCICEPTIVE PROCESSING

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Introduction: Concerns exist over the long term consequences for tail stump pain experienced by piglets after docking, especially in relation to traumatic neuroma development in caudal nerves after docking injury. Neuroma formation may cause detrimental sensory changes in the tail due to altered axonal excitability leading to abnormal sensation or pain.
Aims: To characterize pig tail histopathology at time intervals up to 16 weeks after tail docking and to measure expression of key neuropeptides in caudal dorsal root ganglia and spinal cord neurons associated with (i) peripheral nerve regeneration; activating transcription factor-3 (ATF3), (ii) inflammatory pain; Calcitonin gene-related peptide (CGRP) and (iii) the maintenance of chronic pain; N-methyl D-aspartate (NMDA) ionotropic glutamate receptor subtype 2B (GRIN2B) at the same time points after tail docking injury.
Materials and Methods: Thirty-two female piglets (Landrace/Large White x synthetic sireline) were used (16 docked/16 sham-docked). Piglets were tail docked (amputation of approx. 2/3 of the tail) on post-natal day 3 using a gas hot docking iron. Equivalent sham-docked piglets served as intact controls. Pigs were euthanized by barbiturate overdose 1, 4, 8 and 16 weeks after sham/tail docking. Tail stumps (2 cm) were collected post-mortem for histopathological assessment. Caudal dorsal root ganglia (Ca_1-Ca_4) and associated spinal cord were collected for gene expression analysis by real-time quantitative PCR of mRNA.
Results: Non-specific epidermal and dermal changes associated with healing were observed after tail docking. Mild inflammation, ulceration and oedema were present at 1 week. Traumatic neuroma development was a consistent feature from 4 weeks after tail docking. Neuroma axonal dispersion in the tail stump was on-going 16 weeks after tail docking. ATF-3 mRNA was significantly upregulated in caudal DRGs up to 8 weeks after tail docking, but did not differ at 16 weeks compared with sham controls. Both CGRP and GRIN2B mRNA expression was significantly upregulated 1 week after tail docking in caudal spinal cord neurons but were not significantly different from sham-docked pigs thereafter.
Conclusion: Histopathological lesions that occur shortly after tail docking (beyond 1 week) are not likely to induce or maintain pain. The effects of tail docking on peripheral nerve axonal proliferation and dispersion are relatively short-lived and, although still present, are attenuated by 16 weeks after tail docking injury. Changes in peripheral and spinal nociceptive processing associated with possible inflammatory and chronic pain appear to resolve by 4 weeks after tail docking injury.

Disclosure of Interest: None Declared

Keywords: neuroma, pain, tail docking

Poster Abstracts

Welfare and Nutrition

PO-PC02-020

Swine Inflammation and Necrosis Syndrome (SINS) – a new syndrome related to tail biting in pig

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Introduction: The aetiology of tail biting is highly complex and it´s appearance in a herd can hardly be predicted. Thus, it becomes extremely important to recognise that tail biting is not a singular symptom. Cases of primary cannibalism are rather rare and need differentiation from cases of tail necrosis without support of other pigs, and from cases of secondary cannibalism as a result of primary necrosis of the tail. The aim of the present study was to show that tail necrosis can occur without biting or manipulating the tails and that these signs are part of a syndrome that involves also ears, teats and even claws, heels and coronary band of pigs.
Materials and Methods: We have developed a scoring system to evaluate the degree of inflammation and necrosis of tails, ears, teats and claws in the field. This system has been applied to a set of 44 weaners, kept under standardised conditions on slatted plastic floor for a detailed evaluation of interactions between inflammation and necrosis at the different tissues. The pigs were observed thoroughly and there were neither cases of tail biting, nor cases of claw lesions directly due to problems with the slatted floor (e.g. panaritium).
Results: Swelling of the heels, layering of the claw wall, exudations from the tail and scabs at the tip of the tail were found in 90 to 100% of the pigs. Haemorrhages of the claw wall, reddening and bulging of the soles, heel-sole cracks, congestions of veins of the legs and the ears and rhagades of the tail were visible in 60 to 80% of the pigs. Around 40 to 50% of the pigs had tail necrosis, inflammation of the coronary band and swelling and scabs at the teats. Haemorrhages of the tails and ear necrosis were seen in 30% of the pigs. Ring constrictions of the tails were seen in 5% of the pigs. Tail inflammation and necrosis was significantly correlated with reddening, bulging and haemorrhages of the heels and claw walls, with swelling and scabs at the teats and with venous digestion of the limbs. Claw lesions were found not only in one claw per animal, but in most of the claws; almost all animals were involved.
Conclusion: We conclude that tail necrosis can occur independently (or prior) to tail biting, integrated into a syndrome of correlated lesions at ears, teats and even claws/heels. Facts on the patho-mechanisms of primary necrosis, including aspects of metabolic overload, of endo- and mycotoxins, inadequate feeding, water supply and thermo-regulation are discussed. We point out that the neglection of primary necrosis and the exclusive classification of additional symptoms as traumatic disorders might retard the development of suitable solutions to combat tail biting.

Disclosure of Interest: None Declared

Keywords: necrosis, tail biting

Welfare and Nutrition

PO-PT2-023

Blood plasma replacement by yeast as a source of nucleotides in diet of weaned piglets

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Introduction: The piglets TGI is not yet completely developed after weaning for solid diet digestion and absorption, so different ingredients are used to minimize these problems, improve feed intake and body weight gain during nursery phase. Yeast has been used as an important source of nucleotides, proteins, amino acids, MOS and β-glucans. Based on this, the aim of this study was to evaluate the supplementation of yeast as a source of nucleotides in piglet's diet during nursery phase.
Materials and Methods: 1600 weaned piglets (±21 days of age) Agroceres PIC® were distributed in a randomized block design with 4 treatments and 10 replicates of 40 animals. The nursery phase was divided in 4: pre-initial 1 (22 to 28 d), pre-initial 2 (29 to 35 d), initial 1 (36 to 47 d), and initial 2 (48 to 63 d). The treatments consisted in different inclusions of plasma and *Saccharomyces cerevisiae* yeast as a source of nucleotides [YNU] (Hilyses® - from ICC Brazil Company): 1- Control – conventional diet provided at the farm, with normal levels of plasma (6, 3, 1.5, and 0% - in the respective nursery phases); 2- Diet with plasma reduction (3, 1.5, 0.75, 0) + YNU (6, 3, 1.5 and 0%); 3- Diet with plasma reduction (1.5, 0.75, 0.375 and 0%) + YNU (9, 4.5, 2.25 and 0%); 4- Diet without plasma + YNU (12, 6, 3 and 0%). The piglets begin receiving the experimental diets when they were transferred to nursery facilities after weaning until the end of this phase (±66 days). The feed intake (FI) and body weight were measured at the end of each phase. Based on this, FI (g/d), body weight gain (BWG, g/d) and feed conversion ratio (FCR, g/g) were calculated. The mortality were daily observed and noted. The diarrhea frequency was calculated based on the incidence, intensity and duration, where 0 = no diarrhea presence in any animal; and 100 = 7 days of diarrhea in all piglets. Data were analyzed using the GLM (SAS) and means compared by Tukey (P=0.05).
Results: In pre-initial 1 phase the treatment with plasma (no replacement) resulted in better (P<0.05) FI and BWG, when compared to other treatments. However, during phase pre-initial 2, treatments with the larger proportion of YNU (3 and 4) showed an increase (P<0.05) in FI (26.3 and 13.7%, respectively); in phase initial 1, improved (P<0.05) BWG (30.8%, for both treatments) and FCR (-17.4 and -17.3%, respectively). Considering the total period, the treatment 3 (with low inclusions of plasma, but with YNU) improved numerically (not statistically) FI (4.1%), BWG (8%), FCR (-2.3%), mortality (-83.3%) and diarrhea index (-44.8%).
Conclusion: Plasma can be replaced partially or completely by yeast as a source of nucleotides (Hilyses®), from second week in nursery phase.

Disclosure of Interest: None Declared

Keywords: nursery, performance, Saccharomyces cerevisiae

Welfare and Nutrition

PO-PT2-025

Effects of a phytogenic feed additive on the growth and mortality of nursery pigs

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Introduction: Phytogenics have been considered as alternatives to antibiotic growth promoters (AGPs) and have gained increased attention in feeding programs for swine (Kommera *et al.*, 2006). Special aspects of using phytogenic substances are their beneficial properties on the intestinal tract by modulating gut microbiota and anti-inflammatory effects. Digestarom® P.E.P. is a combination of phytogenics and fructo- oligosaccharides designed to stimulate piglets` appetite through its aromatic properties, optimize digestion and enhance the immune system through its antimicrobial and anti-oxidative effects.
The objective of this trial was to determine the effect of a phytogenic (Digestarom® P.E.P. 125) addition to nursery pig diets on post-weaning growth performance.
Materials and Methods: A total of 180 weaned piglets (6.7 kg BW at 23 days of age) were grown for a 28 days period. Pigs were randomly allocated to one of 3 experimental treatments. Each treatment had 30 pigs per pen and 2 replicates (pens). Data were analyzed using the GLM procedure of SAS with each pen as an experimental unit. Each pen contained one self-feeder and nipple drinker to provide *ad libitum* access to feed and water. The breed used for this trial was Duroc x Yorkshire x Landrace.
Treatment groups were as follows:
Treatment 1: negative control (NC: standard diet)
Treatment 2: positive control (PC: standard diet + Apramycin 100 ppm)
Treatment 3: trial group (PFA: standard diet + phytogenics 125 ppm)
A two-phase diet system was used: Phase 1 diet fed from day 0 to day 14 and Phase 2 diet fed from day 14 to day 28 after weaning. Pigs and feeders were weighed on days 0, 14, and 28 post-weaning to calculate average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), and mortality (%) during trial period.
Results: Pigs supplemented with phytogenics had significantly increased ADG ($P < 0.05$), and better FCR ($P < 0.08$) than pigs fed with antibiotics. However, there were no significant differences compared to the negative control (Table 1).
Mortality was reduced in the PFA group, compared to negative and positive control groups, respectively (Figure 1).
Conclusion: Phytogenics have both a flavoring and a biological effect in animals (Steiner T., 2013). The pronounced improvement in feed conversion ratio and mortality rate in pigs supplemented with phytogenics under commercial conditions indicate the efficiency of this products not only as natural growth promoter but also as a powerful alternative to antibiotic growth promoters in the diet of pigs.

Disclosure of Interest: None Declared

Keywords: growth performance, nursery pigs, phytogenics

Welfare and Nutrition

PO-PT2-026

Selenium transfer to fast-growing pigs from inorganic and organic selenium sources.

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Introduction: Selenium (Se) is an essential microelement for pigs. In pigs, Se deficiency can lead to fatal, degenerative changes in various muscle groups, including heart muscle, compromised boar fertility and increased morbidity due to weakened immune status. Problems related to Se- deficiency seem to re-emerge in feed efficient, fast-growing pigs in Norway. One aim of a large study was to assess the influence of different Se-sources on Se-levels in muscle in Norwegian pigs.
Materials and Methods: Female pigs originating from Landrace*Yorkshire (LY) crossbred sows and Duroc*Duroc (DD) purebred boars (n=24, ~30 kg body weight (BW)) were randomly allocated into eight groups. The pigs were fed eight different diets of pelleted feed supplemented with 100 mg VitE/kg feed. Three different Se-sources were added at varying levels – Sodium selenite (Na_2SeO_3), Se-yeast (Sel-Plex®, Alltech), and selenomethionine (SeMet, Excential Selenium4000®, Orffa). One group received a diet without Se-supplementation (control). Two biopsies from *M. longissimus dorsi* (MLD) were taken during the study using a biopsy-pistol, before initiating the feeding trial and at ~70 kg BW. Samples from MLD were also collected after slaughtering. Total Se-concentrations in MLD and feed samples were measured using inductively coupled plasma mass spectrometry (ICP-MS) and are presented as mean±SD.
Results: The mean Se-concentration in the control diet was 0.05±0.004 mg/kg. The diets supplemented with Na_2SeO_3 contained 0.21±0.01, 0.33±0.006 or 0.36±0.02 mg Se/kg feed. Organic Se supplemented feed contained 0.32±0.00 mg Se/kg (Sel-Plex), and 0.32±0.02, 0.48±0.03, and 0.59±0.03 mg Se/kg (SeMet), respectively.
ICP-MS-results from MLD samples showed an initial Se-concentration of 0.32±0.02 mg Se/kg at 30 kg BW. The Na_2SeO_3 supplementation led to final mean MLD concentrations of 0.29±0.03 mg Se/kg in the low supplemented group, and 0.34±0.02 mg Se/kg in the high supplemented one, respectively. In the Se-yeast-supplemented group, the final mean Se levels in MLD were 0.56±0.02 mg Se/kg. The SeMet-supplemented pigs showed final Se-MLD levels of 0.85±0.04 mg Se/kg (low Se-supplementation), and 2.07±0.15 mg Se/kg (high Se-supplementation). Samples from the control group showed decreased Se-levels at the time of slaughter (0.18±0.01 mg Se/kg).
Conclusion: When comparing with controls and Na_2SO_3-supplemeted animals, high Se-retention was observed in MLD from pigs receiving organic Se. Thereof, pigs receiving SeMet-supplemented feed showed the highest Se concentrations in MLD collected at the abattoir. Further analyses should demonstrate if Se supplementation at different levels with various Se-sources modulates the health condition in Norwegian pigs.

Disclosure of Interest: None Declared

Keywords: Selenium, pig, selenomethionine

Poster Abstracts

Welfare and Nutrition

PO-PT2-029

Digestibility of proximate nutrients and standarized ileal digestibility of amino acids in pigs fed ensiled soaked cowpea (Vigna unguiculata) grains

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Introduction: Tropical native legumes are an alternative to cost-intensive conventional feedstuffs. However, the adverse climate in the tropics induces pest and diseases during storage, affecting the nutritional quality of the feedstuff. Ensiling can face this problem and moreover improve digestibility or reduce the content of anti-nutritional factors (ANF). This study inquired the effects of ensiling a mixture of soaked cowpea (CWP) and sorghum (SOR) grains on fermentation quality, contents of individual ANF, the apparent digestibility (AD) of proximate nutrients (PN) and the standardized ileal digestibility (SID) of selected amino acids (AA) in pigs.
Materials and Methods: Ripe CWP was soaked (24 h) at a grain:water ratio of 1:4 (w:v), drained, milled and mixed with coarsely ground SOR (4 mm mesh size). Molasses (4 %) and lactic acid bacteria inoculant (*Lactabacillus plantarum,* DSM 8862 and 8866, $3x10^5$ cfu) were applied as silage (SL) additives and the homogenized mixture was ensiled (60 d) in plastic tons (120 L). Furthermore, a not ensiled raw mixture (RM) of grains was prepared. Six castrated pigs were allotted in a 3 x 3 Latin square design, two animals per group with an initial bodyweight of 49 ± 2.1 kg. The AD of PN of RM and SL was determined in accordance to GfE (2005). Furthermore, eight adult castrated minipigs with end-to-end ileo-rectal anastomosis were placed in two 4 x 4 Latin square designs. The SID of RM and SL was determined by a regression method (GfE, 2005) restricting CWP inclusion to 0, 10, 20 and 30 % of dietary dry matter (DM).
Results: Differences in the PN composition were only marginal, except for a remarkable reduction of starch from 620 (RM) to 312 (SL) g kg^{-1} DM. Ensiling reduced condensed tannins (CT) from 0.24 to 0.15 % DM. Likewise trypsin inhibition activity (TIA) decreased from 39.6 to 31.1 mg trypsin inhibited g^{-1} DM. Ensiling enhanced ($P < 0.05$) AD of crude ash, ether extract and acid detergent fibre, whereas AD of neutral detergent fibre decreased. AD of crude protein remained unaffected ($P > 0.05$). The SID of N and the majority of AA tended to be higher in SL than RM, except for lysine ($P > 0.05$). However, only SID of methionine was significantly ($P < 0.05$) increased from 56.5 % in RM to 70.3 % in SL.
Conclusion: Ensiling caused a remarkable increase in the digestibility of crude ash, whereas the digestibility of organic matter remained fairly unaffected. The contradictory effect on the digestibility of fibers needs to be clarified further. The decrease of CT and TIA likely contributed in elevated ($P < 0.05$) SID of methionine. The present results indicate that ensiled CWP and SOR mixtures are a suitable pig feed.

Disclosure of Interest: None Declared

Keywords: None

Welfare and Nutrition

PO-PT2-031

Identification of sow level risk factors on early piglet mortality in Danish organic sow herds during different seasons

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Introduction: An 8 year old study showed a high mortality rate with one of three organic piglets dying before weaning in Danish organic sow herds. The high mortality rate constitutes a major economic and animal welfare problem in organic pig production. Several studies have evaluated risk factors for piglet mortality within loose housed and crated sows but there are only limited studies of piglet mortality in the organic production system. In general piglet mortality is expected to be highly multi-factorial. To estimate piglet mortality levels with a high external validity it is necessary to study piglet mortality in commercial organic pig production. The objective of this study was to identify season, litter size, sow parity and sow health as risk factors for still birth and early piglet mortality in Danish organic pig herds.
Materials and Methods: The study was an observational prospective study and included observations performed in nine medium to large Danish organic pig herds over a one year period from June 2014 until June 2015 including approximately 6700 farrowings. Upon transfer to the farrowing field the following information about the sow was recorded: Sow number, parity, body condition and gait score. At the time of farrowing the date was recorded along with litter equalization and the number of piglets that were: live born, still born, small (max. 21 cm), dead after farrowing or euthanized. Counting and registration of the piglets was subsequently performed three times during the seven week long pre weaning period; At castration (or 3-5 days after birth), vaccination (or 14-21 days after birth) and at weaning (7 weeks after birth). If piglets were moved between sows or euthanized during the preweaning period it was recorded. Information about treatments of sick sows including date, condition and use of antibiotics was also recorded.
Results: Preliminary results from seven herds indicate a high mortality rate during summer. The median rate of still birth and early mortality of live born piglets (birth to castration) during the summer 2014 was respectively 8 % and 19 %. Variation between the seven herds however was large ranging from 6 – 12 % in still birth and 11-24 % in early mortality.
Conclusion: Effects of parity, high and low body condition and lameness and litter size will be presented at the conference.

Disclosure of Interest: None Declared

Keywords: Organic pig production, Piglet mortality, Risk factors

Welfare and Nutrition

PO-PT2-036

Isoquinoline alkaloids and naringin improve apparent ileal digestibility and growth performance in post-weaning piglets

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Introduction: Appropriate nutrient digestibility is a key to animal health and growth performance, particularly in post-weaning pigs. Compounds with anti-inflammatory or anti-oxidative properties have potential to support gut integrity and growth performance when included in diets for pigs. Isoquinoline alkaloids (IQ) derived from the *Papaveraceae* plant *Macleaya cordata* exert anti-inflammatory effects, whereas naringin (NRG), a bitter-tasting flavanone glycoside from citrus fruits has anti-oxidative properties. Aim of this study was to determine the effects of IQ or NRG on digestibility and growth performance of post-weaning piglets.
Materials and Methods: Fifty-six barrows and female post-weaning piglets (Danbred, Denmark) with an average initial body weight (BW) of 6.39 ± 0.4 kg and weaned at 25 ± 2 days of age were selected for this 6-wk feeding experiment. Piglets were assigned to 4 treatments according to BW, litter, and gender with 7 replicate pens and 2 piglets per pen. Treatments were 1) Control (basal diet), 2) IQ-1 (basal diet + 60 mg IQ/kg), 3) IQ-2 (basal diet + 120 mg IQ/kg), 4) NRG (basal diet + 50 mg NRG/kg). IQ were provided using a commercial product (Sangrovit® Extra, Phytobiotics Futterzusatzstoffe GmbH, Eltville). Piglets had *ad libitum* access to mash feed and drinking water.
Piglets were fed a starter (25-38 days of age) and grower diet (39-66 days of age) based on corn, soybean meal, wheat and barley. Faecal scores and growth performance were determined weekly. Apparent ileal digestibility (AID) on day 66 days of age was determined using TiO_2 (5 g/kg) as an indigestible marker using one piglet per pen.
Results: Weekly faecal scores did not differ between treatments ($p>0.05$), indicating no major health issues. IQ-1, IQ-2 and NRG improved weight gain in the first two weeks of the experiment ($p<0.05$), whereas FCR was improved in the entire experimental period (Control: 1.47, IQ-1: 1.43, IQ-2: 1.40, NRG: 1.42; $p<0.001$). In addition, overall weight gain was highest and different from the Control in pigs fed NRG ($p=0.023$). Compared to the Control IQ-2 improved ($p<0.05$) AID of protein, phosphorus (P) and total and various single amino acids, whereas NRG increased ($p<0.05$) AID of P, alanine, aspartic acid and leucine.
Conclusion: Supplementation of diets with IQ and NRG increased amino acid digestibility, indicating an increased capacity for nutrient absorption in post-weaning piglets. Increased digestibility was reflected in improved feed efficiency. As such, IQ and NRG represent tools to support gut integrity and growth performance in piglets.

Disclosure of Interest: None Declared

Keywords: Digestibility, Isoquinoline alkaloids, Naringin

Welfare and Nutrition

PO-PT2-040

Effect of lactation stage, parity and body condition on the nutritional and amino acid composition of sow colostrum and milk

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Introduction: Previous research showed that one out of three sows produces insufficient colostrum and milk for the offspring. This may lead to reduced piglet performance. The most obvious solution is to increase the colostrum and milk production of the sow. However, optimizing the composition of sow colostrum and milk may also be a valuable preventive measure. Therefore, knowledge of the composition of the mammary secretions and influencing factors is important. The aim of this study was to investigate the effect of lactation stage, parity and body condition (BC) on the composition of sow colostrum and milk.
Materials and Methods: Fifty sows (parity 1-10) from one commercial pig herd were observed from D0 to D10 of lactation. Colostrum and milk samples were collected at D0, D3 and D10. Nutritional composition (fat, protein, lactose, dry matter) was determined with a Fourier Transform Infrared (FTIR) milk analyzer. Amino acid (AA) were analyzed by an AA analyzer after protein hydrolysis and immunoglobulin G (IgG) by a quantitative sandwich ELISA. Back fat was measured at D0, D3 and D10. Sows were divided into 3 groups based on parity (1, 2-4, >4) and into 3 groups based on back fat thickness at farrowing (<13 mm, 13-16 mm, >16 mm). Repeated measures ANOVA was used to determine the effect of lactation stage, parity and BC, and to assess interactions between lactation stage, parity and BC.
Results: Lactation stage had a significant effect on the nutritional composition and the IgG level of colostrum and milk. This was most pronounced in the first days of lactation (D0-3) with an increase of the fat and lactose content, and a decrease of the protein content and the IgG level. Parity and BC at farrowing had no significant effect on these components. Lactation stage had a significant effect on the AA concentrations and the AA proportions (concentration of each AA / total AA concentration) of colostrum and milk. Sows with a back fat thickness of 13-16 mm showed higher concentrations of Asp, Ser, Gly, Ala, Val, Tyr, Phe, Lys and Arg. Parity had a significant effect on the AA proportions of Ser, Ala, Met, Lys, and Arg. Mainly parity 1 sows differed from the older sows.
Conclusion: Apart from lactation stage, the AA composition of sow colostrum and milk varies with parity and BC. As the neonatal piglet has specific AA needs, part of the effect of parity and BC on piglet performance might be due to changes in the protein quality of colostrum and milk.

Disclosure of Interest: None Declared

Keywords: None

Poster Abstracts

Welfare and Nutrition

PO-PT2-041

An epidemiological assessment of whether the use of high concentrations of zinc oxide in nursery pig diets is associated with post-weaning anemia

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Introduction: Newly weaned pigs require 50-100 mg/kg of zinc in feed to meet dietary requirements (National Research Council). The addition of high levels of zinc oxide (>2000 mg/kg) in nursery diets is often used as a preventive measure for *Escherichia coli* diarrhea and to improve growth performance. The mechanism y which this occurs is still not fully understood, however, it is proposed that zinc oxide has an effect on the gastrointestinal microbiome, due to its bacteriostatic effects. Iron and zinc have similar physical and chemical properties and may rely on the same mechanism for absorption from the gut. An imbalance in the concentration of one mineral can have an antagonistic effect on other minerals. Therefore, the objective of this study is to determine whether high levels of zinc oxide in nursery diets is associated with the presence of anemia 3-weeks post-weaning.
Materials and Methods: Pigs on 20 Ontario (CAN) farms were sampled. Each producer completed a survey answering questions regarding the concentration of zinc oxide in their post-weaning diets and feed tags were collected from these diets to confirm the level of zinc oxide. Each farms was visited twice. The initial visit occurred 1-2 days prior to weaning. Approximately 20 litters per farm were sampled with one small, medium, and large sized piglet selected from each litter (n=1095). An individual body weight and blood sample were taken on the initial visit and then repeated 3 weeks later when the piglets were in the nursery. Hemoglobin was measured using a STAT-Site® M^{Hgb} handheld meter. A mixed logistic regression model was built, with farm modeled as a random effect, and anemia as outcome. Nursery pigs were defined as anemic if their hemoglobin concentration was ≤90 g/L. Hemoglobin status at weaning, the type of iron administered (iron dextran or gleptoferron), and age at weaning were modeled as fixed effects. Zinc oxide concentration in the feed was categorized: nutritional dose (≤500 mg/kg), high dose (2000-3000 mg/kg), and very high dose (>3000 mg/kg).
Results: Zinc oxide concentrations in nursery diets ranged from 250-7000 mg/kg. This study found the odds of anemia was 3.4 and 4.1 times greater for pigs consuming high and very high concentrations of zinc oxide, respectively, compared to those consuming diets containing ≤500 mg/kg of zinc oxide (P<.05).
Conclusion: This study found that high feed levels of zinc oxide (>2000 mg/kg) were associated with a higher odds of anemia in pigs 3-weeks post-weaning. Further research is needed to determine how to off-set the risk of anemia when using zinc oxide to prevent post-weaning diarrhea. Funding: Ontario Pork, the University of Guelph-OMAFRA Research Partnership and Swine Innovation Porc.

Disclosure of Interest: None Declared

Keywords: anemia, swine, zinc oxide

Welfare and Nutrition

PO-PT2-042

Effects of Dietary Palm Kernel Meal with β-mannanase on Growth Performance, Blood Profile, Pork Quality and Economic Analysis in Growing-finishing Pig

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Introduction: International price of swine feed ingredients such as corn and soybean meal has increased continually due to soaring oil price and increasing bio-fuel production. Palm kernel meal (PKM) can be one of alternative ingredients in swine feeds because of its extensive availability, adequate nutrients, comparable price and large amount of production. Therefore, this experiment was conducted to evaluate different levels of dietary PKM with ß-mannanase on growth performance, blood profiles, pork quality and economic analysis in growing-finishing pigs.
Materials and Methods: A total of 120 growing pigs ([Yorkshire × Landrace] × Duroc), average 30.50 ± 3.039 kg body weight (BW), were allotted into each treatment by body weight and sex in 4 replicates with 6 pigs per pen in randomized complete block (RCB) design. The treatments were different levels of PKM in experimental diet (0, 4, 8, 12 or 16%). Blood profiles, pork quality and economic analysis were evaluated. Data were analyzed by analysis of variance using the general linear model procedure of SAS.
Results: In feeding trial, there was no significant difference in growth performance among treatments. However, ADFI was increased (linear, P<0.05) when pigs were fed high PKM diet during the whole experimental period. In BUN concentration, no difference was observed among treatments. The pork pH and proximate analysis of longissimus muscle (LM) were not affected by dietary treatments. In pork color, a* and b* values, were not significant differences among dietary treatments. However, L* value was decreased as dietary PKM level increased. In addition, significant differences were not observed in shear force and water holding capacity (WHC) by dietary PKM with β-mannnanase. Cooking loss was linearly higher when PKM level increased (P <0.05). In fatty acid composition, C16:0, SFA were increased (linear, P<0.05) and USFA, USFA/SFA ratio were linearly decreased as pigs were fed higher PKM treatment diets. The value of TBARS tended to decrease when pigs were fed high PKM treatment diets. When pigs were fed diets containing PKM with β-mannanase, days to market weight was reached earlier compared to basal diet and feed cost was also decreased by supplementation of PKM.
Conclusion: This experiment demonstrated that supplementation of PKM with ß-mannanase in diets of growing-finishing pigs did not show negative responses in growth performance and pork quality. And the highest economical profit was obtained when dietary PKM was supplemented at 8%.

Disclosure of Interest: None Declared

Keywords: growing-finishing pigs, Palm kernel meal, β-mannanase

Welfare and Nutrition

PO-PT2-043

Feeding decisions for the newly weaned pig in east Africa are weight dependent

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Introduction: Smallholder pig farmers in east Africa typically wean pigs at 6 to 8 weeks. Farmers report that commercially prepared pig diets are too expensive and therefore typically feed forage and food waste. This results in low average daily gain (ADG), especially for the newly weaned pig. The objective was to describe the weaning weights of local and crossbred Ugandan pigs purchased from smallholder farmers and to compare the growth rate of the pigs fed forage- or silage-based or commercial diets by their starting weights.
Materials and Methods: Littermate local (n=45) and crossbred (n=45) Ugandan pigs were purchased from 14 smallholder farmers and individually weighed at 9 weeks of age. Pigs were randomly assigned to forage- or silage-based or commercial diets, housed in pens of 3 pigs and weighed every 3 wks. Pigs on each diet were categorized into the lightest, middle, or heaviest tertile. Pig-level ADG was compared within diet and across diet by weight tertile multiple linear regression.
Results: Average (SD) and range of body wt for 9-wk old pigs was 5.7 (1.6) and 2.8-10.2 kg for local and 8.0 (1.8) and 3.9 to 11.4 kg for crossbred pigs. From 9 to 20 wks of age, 19 pigs gained less than 5 kg. All were fed either forage- or silage-based diets. Most pigs on these diets gained less than the smallest pigs fed commercial diet. For pigs fed forage- , silage-based or commercial diets, the ADG of the lightest tertile of pigs was 18, -8 and 154 gm/d from 9 – 12 wks and 115, 142, and 268 gm/d for 18 – 20 wk old pigs fed forage-, silage-based and commercial diets respectively. Similarly, for these 3 diets, the ADG for the heaviest tertile of pigs was 32, 44, and 247 gm/d from 9-12 wks, and 221, 332, and 319 gm/d for 18-20 wk old pigs. The ash levels ranged from 9-12, 12-20, and 9 – 11 % DM for forage-, silage-based, and commercial diets. The high ash levels may in part explain the low ADG in the study. The highest ash levels were found in the first diets fed to the 9 wk old pigs because we included animal-grade dried fish dust rather than human-grade whole dried fish. The ash levels decreased when this change was made.
Conclusion: At 9 wks of age, there was a wide range of weaning weights for local and crossbred Ugandan pigs purchased from smallholder farms. Farmers should be encouraged to feed commercial diet until the pigs reach 11 kg of body weight. Older and heavier pigs grow well on less expensive forage- and silage-based diets. ADG will likely be further improved when the ash content of diets can be reduced from both farmer made and commercial diets.

Disclosure of Interest: None Declared

Keywords: East Africa, forage diet, smallholder

Welfare and Nutrition

PO-PT2-044

EVALUATION OF TWO ANTIMYCOTOXIN AGENTS TO DECREASE THE BIOAVAILABILITY OF ZEARALENONE IN DIETS FOR GILTS.

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Introduction: Zearalenone (ZEA) is a mycotoxin with estrogenic effects, swine being the most sensitive animals, particularly gilts which develop vulvovaginitis and enlargement of mammary glands and the reproductive tract. The objective of this study was to evaluate the toxic effects in gilts that result after consuming 500 µg/kg of ZEA, and the ability of two commercial antimycotoxin agents to reduce the bioavailability of this toxin during a period of 35 days. The product (OA) is an organoaluminosilicate and the product (YA) is prepared based on yeast and algae.
Materials and Methods: 32 recently weaned gilts were selected, and distributed in 4 treatments with 4 repetitions of 2 gilts. The first 7 days were for adaptation. After the 7 days each animal was assigned one of the four experimental diets, which were identified as follows: 1) control diet, with no ZEA or adsorbents 2) intoxication diet with ZEA 500 ppb, 3) challenge diet with 500 ppb of ZEA and 1.5 kg/t of OA, 4) challenge diet with 500 ppb of ZEA + 1.5 kg/t of YA.
Urine samples were collected at days 7, 14, 21, 28 and 35 of the experiment to measure the concentration of ZEA and its metabolites by UHPLC. Before sacrifice, Blood samples from all gilts were collected to obtain serum and perform hepatic profiles, and then they were sacrificed. Samples were collected for histopathological assays. The obtained information was analyzed through the statistical software SYSTAT, by Tukey test where the difference of means was defined. The significance value was based on a $p<0.05$.
Results: Significant statistical differences were found in the weight, and length of the reproductive tract, width of the cervix and vulva volume. In group 3 (OA + ZEA) the effects of the toxin were reduced up to 46%, with respect to the percentage of the reproductive tract weight; but not in group 4 (YA + ZEA), in which more severe effects with respect to the positive control group were observed (ZEA). The histopathological assays confirmed these effects. Quantification of metabolites in the urine showed significant statistical differences between the 4 groups, the highest concentration was detected in group 4 (YA + ZEA).
Conclusion: It may be concluded that the OA product reduces the bioavailability of this toxin because there is a combination in the gastrointestinal tract with a determined quantity of ZEA, while the group with the product based on algae, yeasts etc, did not show the capacity to reduce the bioavailability of this mycotoxin, on the contrary, it improves the adsorption of the toxin in the animal, increasing the ZEA effects.

Disclosure of Interest: None Declared

Keywords: Mycotoxins, zearalenone, organoaluminosilicate

Poster Abstracts

Welfare and Nutrition

PO-PT2-048

Toxic effects of fumonisins in piglets and counteracting strategy

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Introduction: Fumonisins (FUM) are a group of mycotoxins mainly produced by *Fusarium* fungi. They represent a serious threat to swine production as this livestock species is the most sensitive to FUM. They have multiple effects in swine; best described is the impact on pulmonary edema diseases. The aim of the study was to evaluate the negative impact of 50 ppm fumonisins on piglet health and to evaluate a mycotoxin counteracting additive (containing fumonisin esterase).
Materials and Methods: The study was carried out at the Instituto SAMITEC, Brazil. Feed was artificially contaminated with FUM to reach a concentration of 50 ppm. Thirty male piglets were randomly assigned to five groups, six piglets each. Groups were as following: 1) no FUM and no additive, 2) 0.5% additive, 3) FUM, 4) FUM and 0.25% additive, and 5) FUM and 0.5% additive. The trial lasted 42 days, performance data and serum parameters (sphinganine to sphingosine (Sa/So) ratio, total plasma protein) were evaluated on a weekly basis. All data obtained in the experiment were subjected to analysis of variance (one-way ANOVA).
Results: Feed intake was not affected by FUM contamination. Body weight, daily weight gain and FCR were significantly decreased by FUM addition compared to control group. Inclusion of 0.25 and 0.5% additive significantly improved body weight (8.58% and +8.58%, respectively), daily weight gain (+14.06% and +12.50%, respectively) and FCR (-7.81%).
Relative weight of livers and lungs of piglets fed 50 ppm FUM was significantly higher compared to control, +25.89% and 45.65%, respectively. The additive significantly lowered the organ weights of liver and lung compared to FUM group. The total serum plasma protein was not affected. The Sa/So ratio of animals fed 50 ppm FUM was significantly higher (+326.95%) compared to control treatment. The inclusion of 0.25 or 0.5% of additive significantly lowered (-40.86% and -40.70%, respectively) the Sa/So ratio compared with the pigs fed 50 ppm FUM.
Conclusion: A diet containing 50 ppm FUM over a period of 42 days showed deleterious effects in the performance, organ weights and the Sa/So ratio of the piglets. According to the results, the inclusion of 0.25% or 0.50% of a counteracting additive significantly decreased the deleterious effects of 50 ppm of FUM on the evaluated parameters (P≤0.05).

Disclosure of Interest: None Declared

Keywords: Fumonisin esterase, Fumonisins, Mycotoxins

Welfare and Nutrition

PO-PT2-049

Is the relationship between abdominal and rectal temperature constant in healthy and lipopolysaccharide-stimulated, deoxynivalenol-fed pigs?

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Introduction: Body core temperature is an important clinical parameter for monitoring infections in mammals and can be measured with different devices. In practice rectal measurement is the standard method and equated to the body core. In order to investigate the constancy of the relationship between practical body core temperature (rectal) and abdominal temperature under physiological and pathophysiological conditions, we compared both devices in healthy pigs as well as in pigs exposed to LPS and/or DON.
Materials and Methods: A total of 44 barrows were exposed for 4 weeks either to a DON-contaminated (4.59mg DON/kg feed) or a control (CON) diet. They were surgically equipped with an intraabdominal temperature logger (measurement every 5min) and permanent catheters to facilitate infusion with 0.9%NaCl (CON) or LPS (7.5µg/kg BW) either into portal or jugular vein for 60 min. Rectal body temperature was taken every 15min, from 30min before until 180 min after start of infusion. The combination of diet and infusion created six groups: CON_CON$_{jug.}$-CON$_{por.}$, CON_CON$_{jug.}$-LPS$_{por.}$, CON_LPS$_{jug.}$-CON$_{por.}$, DON_CON$_{jug.}$-CON$_{por.}$, DON_CON$_{jug.}$-LPS$_{por.}$, DON_LPS$_{jug.}$-CON$_{por.}$. Data were evaluated by PROC MIXED with group and time as main factors and their interaction and with PROC CORR for method relationship (SAS Enterprise Guide 6.1). Linear regression for both temperature methods was calculated with Statistica (StatSoft, Inc. 1984-2014).
Results: Both methods were significantly correlated in all experimental groups ($p<0.001$). Under physiological conditions both temperatures remained stable during observation period, whereby rectal temperature (38.8°C) was constantly ~1°C lower than intraabdominal measurement (39.7°C). The linear regression showed an increase of 0.68°C intraabdominal per 1°C rectal temperature. Under pathophysiological conditions LPS induced an increase in body temperature ($p<0.01$), starting at 15 min *p.i.* and reaching its plateau at 60min *p.i.*, also with a constant difference of ~1°C. Due to differences in the degree of the rise of the slope the linear regression line is significantly steeper ($p=0.02$) in CON_CON$_{jug.}$-LPS$_{por.}$ (0.97°C) compared to CON_LPS$_{jug.}$-CON$_{por.}$ (0.69°C). Data showed also an impact of DON, with DON_LPS$_{jug.}$-CON$_{por.}$ resulting in ~0.5°C lower intraabdominal temperature compared to CON_LPS$_{jug.}$-CON$_{por}$ ($p=0.08$). Also, the linear regression between both methods showed a steeper slope ($p<0.001$) for DON_LPS$_{jug.}$-CON$_{por.}$ (0.87°C) compared to CON_LPS$_{jug.}$-CON$_{por.}$ (0.69°C).
Conclusion: Rectal temperature does not reflect the actual body core, being constantly ~1°C lower. Also, uniformity of the relationship between both methods cannot be equalized for both, physiological and pathophysiological conditions.

Disclosure of Interest: None Declared

Keywords: body temperature measurement, pathophysiological condition, physiological condition

Welfare and Nutrition

PO-PT2-059

Effects of oral supplementation with probiotics on the performance and gut health of suckling piglets from different genetic lines

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Introduction: The effects of the oral supplementation of probiotics on the performance (weight gain and feed intake) and intestinal histo-morphology of the duodenal, jejunal, and ileal mucosa (villus height, width, and perimeter and crypt depth) of two different genetic lines (purebred Large White or crossbred Large White x Landrace) of suckling piglets.
Materials and Methods: The suckling piglets were evaluated between 2 and 19 days of age. In total, 276 piglets were distributed according to a completely randomized experimental design in a 2 x 3 factorial arrangement, with four replicates each. Treatments consisted of two genetic lines (130 purebred Large White and 146 crossbred Large White x Landrace piglets), two different probiotic products (probiotic bacteria or a combination of yeast and probiotic bacteria), and a control treatment (basal diet with no addition of antibiotics or growth promoters).
Results: Probiotics increased the average daily weight gain (P=0.02), independently of genetic line, but did not influence ($P>0.05$) average daily feed intake. Crossbred piglets presented higher average daily feed intake (P=0.03) than purebreds. The incidence of diarrhea was not significantly different ($P>0.05$) among treatments. Intestinal histo-morphometric parameters were not influenced by genetic lines (P>0.05). However, the piglets fed the probiotic products presented higher duodenal villi compared with the control (P=0.01).
Conclusion: The administration of probiotic bacteria to piglets promoted higher jejunal villus height and lower ileal villus height and perimeter. In addition, modern crossbred genetic lines of pigs may present different nutrient absorption efficiency when comparing to purebred lines. In conclusion, the suckling piglets can be supplemented with probiotics to improve the average daily weight gain, independently of genetic line.

Disclosure of Interest: None Declared

Keywords: Supplement, diarrhea, morphometry

Welfare and Nutrition

PO-PT2-060

Correlation between body weight and stomach capacity in newborn piglets – A pilot study

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Introduction: Increased litter sizes due to hyper prolific sows have led to litters with up to 30% of piglets being born with signs of intra-uterine growth restriction (IUGR) and thereby needing additional care or/and nutritional supplements. Up to 48 % of piglets that die before weaning have empty stomachs, and it is estimated that piglets need at least 250 g of colostrum within the first 24 hours to survive. It has previously been estimated that IUGR piglets only consume 100 g within the first 24 hours and it is likely to increase survivability of these piglets if they are given a colostrum supplement. The aim of this study was therefore to determine stomach capacity of newborn piglets in order to determine how much colostrum supplement can be given.
Materials and Methods: Fourteen piglets that had died on the day of birth were selected. The stomachs were dissected out whole and flushed with water to remove any contents. Stomachs were cordoned off by the exit from the pylorus and then filled with water using a syringe through the pars oesophagea. The stomachs were filled until no more water could be contained without overflowing. The amount of water that had been used (the stomach capacity) was recorded. Data was analysed using SAS and correlations presented as spearman rank correlations.
Results: Piglet weights ranged from 481 g to 1300 g (mean 823 ± 260 g). The average weights of empty stomachs were 4.8 ± 1.14 g, and the average weight of the filled stomachs with water were 36.0 ± 8.6 g. The average volume of water filled into the stomachs was 33.4± 8.8 ml. The smallest stomach capacity was 20 ml and the largest 50 ml. The correlation between stomach capacity and body weight was 0.282 (P=0.329), the correlation between stomach capacity and empty stomach weight was 0.692 (P=0.006). Body weight correlated with stomach empty weight with a coefficient of 0.587 (P=0.027). The stomach capacity of a 1 kg piglet was calculated to be 34.35 ml, however it should be considered that this is an artificial situation and the stomach was inflated to the absolute maximum. It is questionable whether the function of the stomach is maintained when filled to its maximum capacity.
Conclusion: The stomachs could contain from 20 ml to up to 50 ml. There was a stronger correlation between stomach capacity and empty stomach weight than there was between weight and stomach capacity. Currently it would not be recommended to give more than 20 ml of colostrum per kg body weight to newborn piglets in one supplement dosage. Further research is needed on a larger sample size to establish the correct dosages as there was not as strong a correlation with body weight as expected.

Disclosure of Interest: None Declared

Keywords: intra-uterine growth restricted piglets, newborn piglets, stomach capacity

Poster Abstracts

Welfare and Nutrition

PO-PT2-061

Coarse barley inclusion in pelleted diets for finishing pigs reduces gastric ulcers without limiting growth or increasing feed conversion ratio

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Introduction: The use of fine ground diets improve technical performance parameters like average daily weight gain (ADG) and feed conversion ratio (FCR). However, the use of fine ground diets is a risk factor for the formation of gastric ulcers in swine, which have a negative effect on animal welfare. The objective of this study is to improve animal welfare by reducing stomach ulcers without negative effects on animal performance parameters like FCR and ADG.
Materials and Methods: At a commercial finishing pig farm (Topigs 50 x Piëtrain; intact boars and gilts; n=880) finishers were allocated to either a control or the treatment group at the start of finishing at 25 kg of weight. Pigs in the control group received commercial fine ground barley and wheat based diets. Pigs in the treatment group received exactly the same diets, with the difference that 15% of the fine ground barley was replaced by coarse ground barley. Feeds were analyzed for nutrients and for particle size distribution (wet sieve analysis). Pigs were weighed at 0, 35, 70 and 105 days of finishing. The amount of feed supplied per pen was measured by a computer controlled dry feed installation and FCR was measured per two adjacent pens (n= 20 per treatment). To asses the effect on gastric ulcers, one batch of pigs was evaluated at slaughter using a 0-7 scoring system of the pars oesophagus building up from a smooth epithelium (0) towards different degrees of parakeratosis (1-3), different degrees in erosions and ulcers (4-6) until stenosis (7). Statistics were done using SPSS and genstat.
Results: In total 99 stomachs (47 control, 52 treatment) were scored and photographed. Stomachs of the trial group had a significant ($P<0.001$, Mann Whitney U) lower severity of stomach ulcers with an average score of 2.64 compared to the control group with a average score of 4.14. Odds ratio for a stomach score <4 was 0.07 ($p<0.005$) for the trial group. Technical performance did not differ between the control and treatment group (FCR 2.47 vs 2.49 $p=0.49$; ADG 869 vs 867 gram/day $p= 0.85$).
Conclusion: Partial replacement of fine ground by coarse ground barley in pelleted diets decreases the amount of stomach ulcers. This is a sustainable way to improve animal welfare of finishing pigs without increasing the use of raw materials resulting in an equal carbon footprint and economical performance.

Disclosure of Interest: None Declared

Keywords: coarse barley, finishing pigs, Gastric ulcers

Welfare and Nutrition

PO-PT2-065

The use of serum beta-hydroxybutyrate to identify anorexia in newly weaned pigs

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Introduction: Weaning piglets in a commercial setting is associated with physiological changes in the small intestine, and behaviour, which may contribute to piglet anorexia. The clinical signs of anorexia include loss in body condition (thinness) and repetitive oral behaviour (chomping). Anorexic pigs exhibit little change in their body weights and are able to maintain their body condition over the first week post-weaning by utilizing fat stores, so it can be difficult to identify anorexia at an early stage. It has been found that newly weaned pigs that don't eat for 48 hours have elevated levels of serum beta-hydroxybutyrate (BHB). The objective of this study was to determine if pigs identified as anorexic using clinical signs were ketotic based on elevated BHB levels, thus confirming that the pigs were not eating. The second objective was to compare serum BHB levels using a handheld meter (Precision Xtra®) and a BHB assay (Rx Monza) performed at a veterinary diagnostic laboratory.
Materials and Methods: A total of 240 pigs were sampled from 8 commercial farms, with 30 pigs per farm. Farm visits occurred 4-7 days post-weaning because this corresponds to when clinical signs of anorexia are typically first observed. Pigs were selected based on observation of abnormal oral behaviour (Chomp; n=80), poor body condition (Thin; n=80), or healthy appearance (Control; n=80). A blood sample was taken from the selected pigs, and pigs with BHB values > 0.1 mmol/L were defined as ketotic. The Kruskal-Wallis test was used to assess whether the mean ranks of BHB values differed among groups (Control, Chomp, and Thin) and among farms for both test methods (a handheld meter or diagnostic laboratory test). A non-parametric receiver operation characteristic (ROC) curve was used to assess a cut-off value for defining ketotic pigs using the Precision Xtra meter when compared to the > 0.1 mmol/L Rx Monza cut-off value.
Results: Thin and Chomp pigs had elevated mean BHB values compared to Control pigs (both $P<0.05$). Also, there were mean BHB value differences between farms ($P<0.05$). However, less than 15% of the pigs selected based on clinical signs were ketotic, indicating that most were eating. A Precision Xtra® BHB cut-off point of ≥0.2 mmol/L was most effective for determining ketosis on-farm. This cut-off value had a sensitivity of 100% and specificity of 96.4%.
Conclusion: This study found that relying on clinical signs alone is not an accurate way to identify anorexic pigs. Thus, it is necessary to use BHB testing to help identify affected animals at an early stage and to gain a better understanding of post-weaning anorexia. This work was supported by Ontario Pork and the Saskatchewan Agriculture and Development Fund (ADF).

Disclosure of Interest: None Declared

Keywords: anorexia , beta-hydroxybutyrate, swine

Welfare and Nutrition

PO-PT2-067

How spray dried plasma can replace antibiotics at weaning: in-vitro effects of plasma on intestinal porcine epithelial cells.

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Introduction: The weaning period is a challenging time for piglets. Their feed intake decreases and about 20% of the piglets suffer from post weaning diarrhoea (PWD). PWD is an important cause of economic loss, due to high morbidity and mortality, decreased growth, and costs for medication. Control of PWD is often done by antibiotics or by inclusion of zinc oxide in feed. PWD is mainly caused by the abrupt loss of protection by maternal antibodies which prevents colonization of the intestine with pathogens like the enterotoxigenic bacterium E. coli F4+ (K88)(ETEC), which is the most common cause of PWD.
Spray dried plasma (SDP) is widely applied in weaning diets to support the milk-to-feed transition of the piglet. Multitudes of studies on plasma show the beneficial effects on piglet performance and indicate that SDP may be a good alternative for antibiotic treatment. In the present study gene expression analyses were conducted to elucidate biological mechanisms by which SDP induces beneficial effects in the intestine of weaned piglets.

Materials and Methods: Cultured porcine enterocytes (Intestinal Porcine Epithelium Cells; IPEC-J2) were incubated with SDP products and controls, and were or were not challenged with ETEC bacteria. The expression level of genes (coding for 14.000 unique proteins) was measured with a microarray. Clusters of genes that were differently expressed were identified. Bioinformatics programs were used to identify biological processes affected by SDP.

Results: The *in-vitro* results indicate that SDP steers a variety of biological processes in the enterocytes of pigs inflamed by ETEC. From the regulated gene clusters it appears that SDP

1) protects against infectional damage and maintains the barrier function of the intestinal epithelial layer;
2) improves alertness (recognition by Toll-like receptors) and function of the immune system;
3) stimulates cell-renewal in the epithelial wall; and
4) tempers Interferon and IL6–induced inflammatory reactions in the intestine which may prevent overreaction of the immune system.

Conclusion: A part of the identified biological processes and effector molecules are similar to those observed after treatment with antibiotics and zinc-oxide. These results indicate that similar mechanisms contribute to the observed beneficial effects and confirm the statement that SDP products are a good alternative for antibiotics in reducing PWD.
The need to reduce antibiotic treatment stimulates the search for alternative management, feeding and treatment strategies. The present study gives a methodological approach to screen for candidate in-feed alternatives and secondly hints to mechanisms whereby SDP supports the gut health around weaning.

Disclosure of Interest: None Declared

Keywords: antibiotics, spray dried plasma, weaned pigs

Welfare and Nutrition

PO-PT2-072

A correlation between tail docking length and formation of amputation neuroma

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Introduction: In pig production, piglets are tail docked in order to prevent tail biting later in life. As known in preceding researches, the amputation neuromas may occur at the docked tail tip that altered nociceptive thresholds which causes lifelong pain to pigs. The aim of this study was to investigate the interrelationship between docked tail length and the formation of amputation neuroma associated with the aspects of animal welfare.
Materials and Methods: A total 300 pig tails were cut off 0.5cm from the tip, and the length and diameter were measured by a ruler. The docked tail length was a distance between 1cm from the root and 0.5cm from the tip, and the diameter was at the 0.5cm from the tip. Cross section was made according to methods by M.S Herskin *et al.*, 2014. . In their research, the intact tail length of crossbred LYD (Landrace x Yorkshire x Duroc) was 30.6 ±0.6 cm (n=65). The sections were examined by Olympus BX50 microscope (Olympus, Tokyo, Japan) at magnifications 4, 10 and 25X. The dimensions of neuromas in the sections were measured by image analysis software (IMTi-Solution Inc., Burlington, Canada). The experimental design was divided into 3 groups according to range of tail length calculated through the referred intact tail length, mentioned above: group L (long, n=58 (19.3%), 15.3 cm ≤ length < 22.95 cm, 25~50% docking), group M (moderate, n=187 (62.3%), 7.65 ≤ length < 15.3, 50~75% docking) and group S (short, n=55 (18.3%), length < 7.65, 75~100% docking). The number of tails with neuromas, the number of neuromas per tail and the mean size of neuromas were measured. Kruskal-Wallis test and Fisher's exact test of SPSS statistics 21 (IBM Corp., USA) were used for interrelationship between docked tail length and formation of amputation neuroma.
Results: The total percentage of tails accompanied with neuromas was 72.3% (n=217), the number of neuromas per tail was 0.84 ± 0.64 and the mean size of neuromas was 1,122.30 ± 378.8㎛, respectively. The results of above variables in each group as follow: group L was 70.69% (n=41); 0.93 ± 0.80 and 1,137.57 ± 401.90 ㎛, group M was 78.60% (n=147); 0.88 ± 0.56 and 1,109.03 ± 368.51 ㎛ and group S was 52.73% (n=29); 0.64 ± 0.68; 1,162.57 ± 394.04㎛, respectively. No statistically significance was observed except number of neuromas per tail (p = 0.017).
Conclusion: This study focused on the relationship between docked tail length and formation of neuromas. Neuromas were found throughout all docking lengths, and the extent of neuroma formation was not affected by docking length.

Disclosure of Interest: None Declared

Keywords: neuroma, pig, tail docking

Poster Abstracts

Welfare and Nutrition

PO-PT2-077

Corn and Finished Feed Multi-Mycotoxin Survey 2015

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Introduction: Mycotoxins are a large family of toxic fungal metabolites which occur worldwide in various cereals and other feed commodities. During the whole chain from field to feeding, mycotoxins can be produced by molds in plant material. There are numerous mycotoxins which are very toxic to farm animals and may cause different diseases. Therefore they are responsible for reducing animal production.
Materials and Methods: To test the occurrence of multiple mycotoxin metabolites, a method based on Liquid Chromatography coupled with tandem Mass Spectrometry was used (LC-MS/MS method, Spectrum 380®). As the sensitivity of this test method has improved, it can test many more mycotoxins (e.g. masked mycotoxins) in addition to the main mycotoxins aflatoxins (Afla), zearalenone (ZEN), deoxynivalenol (DON), fumonisins (FUM), T-2 toxin (T-2) and ochratoxin A (OTA).
In 2015, a total number of 108 corn and 367 finished feed samples were analyzed from over 30 countries around the world.
Results: Among the most common mycotoxin metabolites in finished feed samples found are zearalenone (97%) and the penicillium toxin emodin (96%), detected in nearly all samples at an average of 134 ppb and 33 ppb respectively. Also Type B trichothecenes were detected in a high amount: deoxynivalenol occurred in 92% and nivalenol in 63% of all finished feed samples. In total, 78% of all finished feed samples contained between 30 and 50 mycotoxins and their metabolites. Considering corn samples, the Fusarium metabolites moniliformin (91% occurrence) and beauvericin (90% occurrence) represent the most common toxins. Type B trichothecenes like DON-3-glucosid and nivalenol were detected in 66% and 57% of all corn samples at an average of 261 ppb and 51 ppb, respectively. Two-thirds of all corn samples contained between 20 and 40 different metabolites. Fusarium toxins such as deoxynivalenol, zearalenone and fumonisins occur in all samples. Ochratoxins represent the least occurring mycotoxins; since it only being identified in less than 1% of all tested corn samples.
Conclusion: The multi-mycotoxin LC-MS/MS method offers more detailed results than common testing methods. Looking at the results it can be said that zearalenone is the most occurring mycotoxin in finished feed samples and moniliformin the most frequently occurring metabolite in corn samples. Nevertheless, in total, Fusarium toxins represent the main group of frequently occurring mycotoxins in both types of feed. Though more advanced technologies such as LC-MS/MS allow for more detailed investigation and detection of mycotoxins, more research is needed to assess the effects of these mycotoxins and metabolites on pigs.

Disclosure of Interest: None Declared

Keywords: Mycotoxins

Welfare and Nutrition

PO-PT2-083

Effect of supplemental Hostazym® X xylanase complex on mortality and removal rate of fattening pigs in various feed compositional trials.

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Introduction: Hydrolysis of Non-Starch Polysaccharides (NSP) by xylanase is known to deliver favourable arabinoxylan-oligosaccharides which are fermented into highly absorbable short-chain-fatty-acids. This prebiotic effect may cause a drop in intestinal pH, alter microflora composition in the hindgut and consequently be translated in reduced mortality. The objective of these trials was to identify effects of a xylanase complex on mortality and removal rate of fattening pigs.
Materials and Methods: In 3 different large-scale pig trials (integration system and commercial farm), with varying diet compositions, the effect of xylanase supplementation (Hostazym® X, Huvepharma Inc.; Huvepharma N.V.) on overall mortality and removal rate was evaluated.
Trial 1 (USA, 2014): the control group (n=1335 pigs) and xylanase supplemented group (n=1294 pigs) at 1500 EPU/kg were fed a maize-soy-DDGS-based diet from 11 up to 116 kg of BW.
Trial 2 (Belgium, 2015): the control group (n=256 pigs) and xylanase supplemented group (n=256 pigs) at 1500 EPU/kg were fed a wheat-soy-based diet from 23 up to 112 kg of BW.
Trial 3 (USA, 2015): the control group (n=530 pigs) and xylanase supplemented groups at 1500 (n=530 pigs) and 3000 EPU/kg (n=530 pigs) were fed a wheat-soy-based diet from 11 up to 127 kg of BW.
In these 3 fattening pig trials all animals had continuous access to water and feed. For trial 1 and trial 2, measurements of initial and final BW were compared. The occurrence of mortality and removal was recorded for all 3 trials.
Results: Overall, the xylanase positively influenced pig growth in both trial 1 (0.43% vs. control) and trial 2 (1.17% vs. control). In trial 1 the supplementation of the xylanase complex resulted in a 32.54% lower mortality and morbidity level vs. the non-supplemented control group. A similar trend was observed in trial 2 in which the xylanase treated group showed a 27.91% reduction in overall mortality rate vs. the control. In trial 3 a consistent drop in mortality and morbidity rate was observed from single dose 1500 EPU/kg (-20.06%) to double dose 3000 EPU/kg (-50.14%) vs. control.
Conclusion: This summary of large-scale pig trials consistently demonstrates the effect of the supplemental xylanase complex in overall removal rate, irrespective of diet composition or genetic conditions. This positive effect, linked to a prebiotic interaction of xylanase-mediated hydrolysis products with the gut microflora, was confirmed. This is in full accordance to what was already observed in multiple broiler trials using the same xylanase product. It was concluded that the prebiotic effect of Hostazym® X has a significant impact on overall pig removal rate.

Disclosure of Interest: None Declared

Keywords: fattening pigs, mortality effect, xylanase complex

Welfare and Nutrition

PO-PT2-088

Meat quality parameters in castrated versus vaccinated against gonadotrophin-releasing factor in female Iberian pigs reared in free-range conditions

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Introduction: The Iberian pig is a native breed of Spain used for the production of dry-cured ham. It is typically kept in extensive systems, with a slaughter age ranging from 10 to 24 months. To avoid welfare, sanitary and production issues linked to unwanted pregnancies caused by invading wild boar or co-housed males, gilts were traditionally spayed. However, EU legislation restricts this practice. Vaccination with an anti-GnRF vaccine (Improvac® / Vacsincel®, Zoetis) was tested previously for its ability to suppress estrus and estrus-related behavior in female pigs. The aim of the present study was to compare the effect on meat and carcass quality of vaccination against GnRF in comparison to surgical castration or rearing entire Iberian females in extensive conditions.
Materials and Methods: This study was carried out with 43 animals: castrated (CF; 19), entire (EF; 8), and vaccinated against GnRF (VF; 16) Iberian females reared in extensive conditions until the age of 16 months with a final live weight of 155.7 ± 8.4 kg. The vaccination against GnRF consisted on the application of three doses of 2 mL of IMPROVAC® (Zoetis) intramuscularly at the neck region of each VF pig at 11, 12 and 14 months old. Carcass and meat quality was assessed, including size of the carcass and pieces such as hams and shoulders; pH, texture, colour and fat content of the meat and sensory traits of cooked loins in terms of aroma and taste.
Results: Carcass traits were found to be very similar at the three female groups, although EF had higher ($P = 0.0236$) killing out percentages than VF and the loin area and the percentage in relation to the total that represents the loin and shoulder was highest ($P<0.05$) in VF than in CF (Table 1). Regarding meat quality characteristics, no one of the parameters measured, including pH, colour, texture or fat content were found to be significantly different between the three groups. Concerning the sensory characteristics of meat from the three types of females, significant differences ($P<0.05$) were detected in only 2 of the 16 attributes evaluated. *Longissimus thoracis* from CF showed a higher ($P = 0.012$) overall flavour intensity than meat from VF and EF as well as higher ($P = 0.023$) sweetness than EF. However, the differences between the three types of females are very subtle (14 attributes were scored in the same way and minor numerical differences were found in the other two).
Conclusion: Rearing entire females or vaccinated against GnRF females when entire males (i.e. wild boars) can be in contact with females is a suitable alternative to surgical castrated females in Iberian pigs maintained in free range conditions because there is no differences on technological or sensorial meat quality.

Disclosure of Interest: None Declared

Keywords: extensive production, female pigs welfare, inmunocastration

Welfare and Nutrition

PO-PT2-089

A field study with undocked tails on 15 conventional farms in North Rhine-Westphalia, Germany

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Introduction: Routine tail docking is not permitted in the EU. This is documented in Council Directive 2008/120/EC, which defines the minimum standards of welfare for pigs. Nevertheless tail docking is very common in all countries with a dense pig production as a precaution to prevent tail biting. Farmers' organisations and the ministry of agriculture in North Rhine-Westphalia, Germany signed a voluntary declaration in 2014 to develop other means to avoid tail biting. It was decided to start with keeping of small groups of undocked pigs in conventional pig production.
Materials and Methods: A total of 786 pigs were observed from birth to slaughter in 15 farms. Group size varied from 30 to 94 pigs. The median size per farm was 210 sows, 1000 weaners and 1490 fatteners. In all herds i.e. health, climate conditions, feed components and water supplies and qualities were checked before raising undocked piglets. Subsequently, herd management conditions were optimised to prevent biting. Parts of the trial were open water access and two times a day pigs were fed with a handful hay or corn-silage. The farmers were trained to register tail biting marks. Once a week, an external veterinarian examined the undocked pigs. In the case of tail-biting additional enrichment material, raw-fibre feed supplement, salt licks with minerals or a lick with molasses were given.
Results: In the compartments with undocked pigs, farmers needed more time for observation and handling biters and bitten pigs. In case of tail biting, it was very important to change the enrichment material more often. On 3 farms, tail biting was already observed before weaning. From a total of 725 piglets 22 (3%) showed lesions and 3 (0.3%) tail losses. After weaning, the median frequency per group and farm was: For necrosis 3.9%>54.3% (6 farms), for lesions 3.3-46.7% (8 farms), for tails with blood 1.9-14.6% (9 farms) and for partial losses of tails 2.0-51.0% (11 farms). At the end of the weaner period we observed 578 (73.8%) weaners with entire tails and 50 (6.3%) weaners with small lesions and 158 (20.1%) with tail losses. Only on two farms, all undocked pigs retained entire tails until the beginning of the fattening period. By now the finishing period is not completed.
Conclusion: Deficiencies in food components and water supply are important triggers for tail biting. The farmers need more time to observe the undocked pigs and for treatment of biters and injured pigs. Changing enrichment material more frequently is more effective than the quantity of the material. Early identification of biters is the crucial step to stop tail biting. Until now, raising undocked piglets in conventional pig production is only recommended in small groups to acquire experience.

Disclosure of Interest: None Declared

Keywords: field study, tail biting, tail docking

Poster Abstracts

Welfare and Nutrition

PO-PT2-091

Individual and combined effects of subclinical doses of mycotoxins deoxynivalenol and fumonisins on the vaccinal and intestinal responses of piglets

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Introduction: Mycotoxins are a group of structurally diverse secondary metabolites of fungi that can result in health problems in animals and severe economic losses. Pigs are naturally exposed to mycotoxins due to the high inclusion of cereals and by-products in their diets. Based on recent surveys, 70% of worldwide feed and feed raw materials are positive for at least one mycotoxin and 40% are found to be co-contaminated. Co-occurrence of mycotoxins is thus the norm and not the exception, and until now not much is known about the risk in pigs of exposure to multi-contaminated feed.
Materials and Methods: Therefore, we investigated the interaction between deoxynivalenol (DON) and fumonisins (FB), two major mycotoxins from *Fusarium* species co-occurring very often in finished feed. Piglets received separate diets for five weeks: a control diet; a diet contaminated with either DON (3 mg/kg) or FB (6 mg/kg); or both toxins. The levels of contamination were considered representative of field conditions. Given the mode of action of both mycotoxins, the effects on the vaccinal response were investigated following immunization of pigs with ovalbumin (OVA), as well as the effects on the intestinal response.
Results: Pigs fed DON and/or FB were not able to mount an appropriate immune response following vaccination, especially in pigs fed the co-contaminated diet, as seen with the reduced concentration of specific anti-OVA IgG, the low proliferation of lymphocytes upon OVA stimulation, and the decreased expression of cytokines in the spleen (IL-1β, IL-8, IL-6, IL-12p40). Our findings in the small intestine (jejunum and ileum) also suggested that ingestion of DON and/or FB affected the gut integrity and induced intestinal alterations that are similar to those observed during low-grade inflammation and chronic disease (up-regulation of pro-inflammatory cytokines, reduced expression of tight junction proteins, and infiltration of immune cells). The type of interaction between DON and FB was determined by analyzing the data with a two-way factorial ANOVA on each endpoint assessed. The interaction of DON and FB was very dependent on the endpoint, with ten endpoints reporting antagonism, eighteen additivity, and three synergism.
Conclusion: In conclusion, ingestion of subclinical doses of mycotoxins does not induce visible clinical signs but result in immunological disturbances and may act as predisposing factors and impair the response of animals to antigenic challenges, such as vaccination or infection. In addition, further work is needed to characterize the type of interaction between mycotoxins and evaluate the risk in animals since current legislations in feed only set limits for one single mycotoxin.

Disclosure of Interest: None Declared

Keywords: Interaction, Mycotoxins

Welfare and Nutrition

PO-PT2-092

Offering different roughages to fattening pigs: ingested amount and effects on gastric health

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Introduction: Gastric ulcerations are a special challenge in modern pork production. Stress can be a reason but in particular the existing milieu conditions have an impact on the development of gastric lesions. The dietetic effects of fibre sources in pigs' gastrointestinal tract are well known. In some cases fibre sources are used as material for the pigs to root and manipulate. The present study was focused on the amount ingested when fibre sources are offered and whether there is an influence on gastric health.
Materials and Methods: In total 170 weaned pigs (bw: 27.0±2.79 kg) were housed individually and had free access to water. A commercial pelleted diet (ME: 13.5 MJ/kg as fed, XP: 17.2/16.8/15.9 %, XF: 3.8-4.4 %) was fed in three phases. Eight groups were formed in which different types and amounts of additional crude fibre source were offer: control (C)/15 g straw (S)/15, 50, 100 g straw (S+)/15, 50, 100 g straw pellets (SP)/10, 30, 50 g palm kernel meal (P)/30, 75, 100 g palm kernel meal (P+)/100, 175, 200 g whole plant corn silage (CS) or 200, 400, 600 g whole plant corn silage (CS+) in the respective phase. Wet sieve analysis of the pelleted diet was performed. Feed intake of fibre source was recorded over the whole fattening period. After feed was withheld overnight pigs were slaughtered (contemporarily after leaving farm) at the end of fattening (bw: 121±3.88 kg) and stomachs were examined: The mucosa of the pars nonglandularis was scored macroscopically with a five step score system.
Results: The fraction of particles >1.00 mm was 38.1, 39.1 or 28.7 % and the amount of particles smaller than 0.20 mm reached 29.9, 31.2 or 39.2 % in the diet of 1st, 2nd or 3rd phase of fattening. During the entire fattening period the intake of fibre sources (S/S+/SP/P/P+/CS/CS+) varied with 0.410/0.632/1.79/1.23/1.74/5.79/8.59 kg as fed. Compared to the totally offered amounts, the consumption of fibre source reached 26.0/8.67/24.2/31.5/21.2/32.7/17.6 %. Especially in SP the amount consumed varied highly. Non single animal in any group ingested more than 57 % of the offered fibre source. Independent of the groups and the fibre consumption, 95 % of the animals were affected by marked stomach lesions (erosion, ulceration).
Conclusion: The uptake of corn silage was highest compared to the other fibre sources indicating that palatability influences the amount of ingested roughage. No desired prophylactic effect of roughages on gastric health. This investigation did not indicate any desired effect of fibre source or amount of consumption on gastric health. Having a huge amount of fine particles in the diet the additional offer of roughage cannot prevent the deficiency in gastric health.

Disclosure of Interest: None Declared

Keywords: fattening pigs, gastric health, roughages

Welfare and Nutrition

PO-PT2-095

Intradermal vaccine application: effects on suckling piglet behaviour

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Introduction: Intradermal (i.d.) application of vaccines offers an improved hygienic standard compared to intramuscular (i.m.) injection. While previous studies confirmed similar immune responses in animals vaccinated i.d. and i.m., studies on consequences on animal behaviour are still lacking. Therefore, this study evaluated welfare aspects of i.d. and i.m. vaccination in suckling piglets.
Materials and Methods: The study was carried out in three batches in a commercial German pig farm. In the test group, 338 piglets were i.d. vaccinated with Porcilis® M Hyo ID ONCE with the IDAL injector (MSD Animal Health) on the 8th day of life. In the control group, 334 piglets were vaccinated i.m. according to standard farm procedures. On the following three days, the injection site was evaluated with regard to size of swelling and rubor. Piglets were weighed individually and body-temperature-measured one day before vaccination and eight days later. For ten days, starting two days before vaccination, continuous video recordings were performed in order to assess piglets' resting, activity and suckling behaviour as indirect parameters of stress and pain. Six litters vaccinated i.d. and six control litters were observed. Video analyses via scan sampling in 5 minute-intervals concentrated on the day before vaccination, day of vaccination and day after vaccination. All data was statistically analysed using the software IBM SPSS Statistics, version 22.
Results: Daily weight gain did not differ significantly between piglets vaccinated i.d. (247 g/d) and those vaccinated i.m. (258 g/d). There was no significant difference in body temperature between groups. On the first day after vaccination, test group piglets had more swellings than those in the control group. However, this difference disappeared by the seventh day after vaccination. On the day before vaccination, piglets in the test and control group differed significantly only in their standing behaviour (test group: 27.0% vs. control group: 20.1%). On the day of vaccination, piglets vaccinated i.d. lay significantly less frequently (70.2% vs 82.1%) and showed significantly more walking (14.0% vs. 11.7%) and suckling (46.0% vs. 35.6%) behaviour. On the day after vaccination, these effects were still observed for walking (18.1% vs. 17.2%) and suckling (48.4% vs. 36.4%).
Conclusion: Similar to other studies, piglets stressed or suffering pain show altered behaviour patterns, for instance less suckling activity. Therefore, the results of this study indicate a reduced degree of stress in suckling piglets after i.d. vaccination in comparison to i.m. vaccination.

Disclosure of Interest: None Declared

Keywords: Suckling behaviour, video analysis, welfare

Welfare and Nutrition

PO-PT2-096

A porcine pain face –identifying visible characteristics of pain in pigs

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Introduction: Clinician and pain researchers agree that there is a need for measures of pain. In pigs, being a species of prey, pain may be concealed and thus difficult to assess. However, identification of pain is necessary to enable its alleviation. Several physiological and behavioural parameters have been used in this respect during the last decades, however with only little success. A useful method must be able to distinguish pain-related changes from behaviours related to *e.g.* drowsiness or diseases. The pain face, *i.e.* the description and measurement of changes in facial expression related to pain, have been considered as such a method in several species including man.
Materials and Methods: Topical capsaicin crème has previously been used for the experimental induction of a short action, reversible inflammatory type pain in horses and man. In this study, capsaicin was used aiming to develop a repeatable and reliable model for pain induction and the description of a porcine pain face. Following the dermal application of capsaicin, six pigs were video recorded. Each pig served as their own control, and was filmed with and without noxious stimulus in separate trials. All trials were performed with and without an observer and with the crème applied on the left respectively the right shoulder, in total six recordings per pig. Based on characteristic facial expressions in other species, qualitative changes of eye region, ear basis, snout and cheeks were described by two external assessors and a drawing of a porcine pain face was made. Subsequently, 126 still images were evaluated blindly to assess the presence or absence of these features. Finally, an ethogram was constructed to describe certain gross pain behaviours observed.
Results: The facial expressions of the porcine pain face comprises an angled appearance of the "eyebrows", lowered ears held back or in an asymmetrical manner, wrinkling of the snout and tension of certain muscles around the mouth and cheeks. In the blinded evaluation, an "angled eye" was significantly (P=0.004) correlated to the application of a noxious stimuli whereas the number of wrinkles of the snout were not (P=0.8). Among the gross behaviours assessed, "up and/or attentive positioning" of the ears were correlated (P=0.02) to control trials.
Conclusion: The facial expressional changes observed in the pigs during noxious challenge were subtle. However, this study indicates that a porcine pain face can be identified and may be developed to constitute a method for the assessment of pain. Further development is needed to establish the optimal concentration of the crème for various ages of animals and methods to obtain video recordings from standardized positions.

Disclosure of Interest: None Declared

Keywords: capsaicin, ethogram, pain face

Poster Abstracts

Welfare and Nutrition

PO-PT2-101

Effects of Wheat Supplementation Level on Growth Performance, Blood Profiles, Pork Quality and Economic Analysis in Growing-finishing Pigs

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Introduction: There are many proper alternatives that can be used to meet the nutritional requirements of swine while reducing feed cost. Wheat has a higher content of crude protein and lysine than those of corn. In addition, many researchers reported that wheat could substitute for corn in growing-finishing pig without negative effects on growth performance and improve better carcass than pigs fed corn (Han et al., 1976; Bell and Keith, 1994; Han et al., 2005). Therefore, this experiment was conducted to evaluate different levels of wheat as alternatives to corn on growth performance, blood profiles, pork quality and economic analysis in growing-finishing pigs.
Materials and Methods: A total of 120 growing pigs ([Yorkshire × Landrace] × Duroc), average 27.75 ± 6.391 kg body weight (BW), were allotted into each treatment by BW and sex in 4 replicates with 6 pigs per pen in randomized complete block (RCB) design. The experimental treatments were 0, 15, 30, 45 or 60% of wheat in corn-SBM diet. Four phase feeding programs were used in this experiment. Blood profiles and pork quality were evaluated. Economic analysis was calculated using amount of the total feed intake and feed price. Data were analyzed by analysis of variance using the general linear model procedure of SAS.
Results: During whole experimental period, there was no significant difference in growth performance among treatments. However, G:F ratio tended to increase (quadratic, $p<0.08$) when pigs were fed diet higher levels of wheat during finishing period. No differences were observed in BUN, creatinine and total protein concentration among treatments. The proximate analysis of pork was not affected by dietary treatments. However, pH of pork was decreased as dietary wheat level was higher in 0 and 6 hours (quadratic, $p<0.05$). Pork and fat color, L*, a* and b* values, shear force and WHC, cooking loss had no differences significantly among dietary treatments. When pigs were fed diets containing wheat, days to market weight was numerically reduced compared to corn-SBM basal diet.
Conclusion: This experiment demonstrated that supplementation of wheat did not show negative responses on growth and carcass characteristics. Moreover, dietary wheat supplementation improved G:F ratio during finishing period and economical profit was increased by reduction days to market weight compared to corn-SBM control treatment. Consequently wheat can be supplemented up to 60% in growing-finishing pig's diet without detrimental effects on growth and pork quality.

Disclosure of Interest: None Declared

Keywords: growing-finishing pigs, Wheat

Welfare and Nutrition

PO-PT2-104

Bacterial microbiome in colon and faeces of weaned piglets in dependence of varying phosphorus levels in the diet

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Introduction: Phosphorus is an element with potential impact on the intestinal microbiota of pigs. Studies revealed that bacterial growth was limited in the intestine feeding low concentrations of phosphorus and that ileal bacteria increased with higher calcium and phosphorus feed contents, whereas there was no effect on bacterial numbers in the colon. Therefore, the aim of this study was to evaluate the influence of varying phosphorus levels in diets of piglets on the bacterial microbiome in colon chyme and faeces.
Materials and Methods: Weaned piglets (n=18) were divided into 3 groups (n = 6) fed a pelleted diet (on the basis of wheat, barley and soybean; supplied with varying levels of monocalcium phosphate) with soluble phosphorus contents of 0.32% (Low-P), 0.54% (Normal-P) and 0.74% (High-P) of dry matter. On day 35 (d35) samples were taken from each piglet before slaughtering (faeces) and after slaughtering (intestinal chyme). Bacterial DNA was isolated and amplificated *via* polymerase chain reaction. Afterwards, a denaturing gradient gel electrophoresis (DGGE) was performed and sequencing was conducted by Eurofins (Ebersberg, Germany). Sequences were compared with the database of the National Center for Biotechnology Information using the Basic Local Alignment Search Tool. The DGGE band patterns were analyzed with Bionumerics 5.0 (Applied Maths, Inc., Sint-Martens-Latem, Belgium).
Results: Dietary P-level neither affected DGGE band patterns nor the appearance of species in the different diet samples. Less than 60% similarities between band patterns of repeated trials were found, whereby band patterns of colonic and faecal samples of each experiment showed similarities of up to 80%. Dominating species in colonic and faecal samples were *Roseburia faecis*, *Eubacterium cellulosolvens*, *Sarcina ventriculi* and *Butyrivibrio fibrisolvens,* which are involved in the bacterial degradation of indigestive polysaccharides (except *Sarcina ventriculi*). Facultative pathogens (e. g. *Streptococcus gallolyticus*) were rarely detected and could not be identified in Low-P. *Lactobacillus* spp. was documented in colonic and faecal samples of solely one piglet.
Conclusion: Using DGGE, no clear effect of the dietary P-level on the colonic and faecal bacterial microbiome was detected and there was no apparent effect of the diets on the occurrence of species or DGGE band patterns. Assuming that there is no significant impact on the intestinal microbiome a P-reduction in swine nutrition might be feasible without negative effects on the intestinal health.

Disclosure of Interest: None Declared

Keywords: None

Welfare and Nutrition

PO-PT2-108

Feeding behaviour and performance in relation to injurious tail biting in boars - a longitudinal study

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Introduction: Automatically collected feeder data may be used to predict tail biting in finisher pigs.
Materials and Methods: Pen-level feeding behaviour and growth were investigated in relation to injurious tail biting (ITB), defined as visible wounds, from 10 weeks before to 4 weeks after the first ITB case in the pen. The data set included 36 pens of 10-12 intact boars between 43 and 148 kg, with average pen weight at ITB onset between 78 and 137 kg. A tail biting pen (TBPEN) had at least one case of ITB, whereas a control pen (CTR) had none. Individual feeding-related data including consumed feed, bout length and -frequency were collected by a single automatic ad libitum feeder. Time (week) relative to ITB onset was referred to as RELWEEK. The time before (PRE-ITB, RELWEEK -10 to 0, n=13 TBPEN and 23 CTR pens) and after ITB onset (POST-ITB, RELWEEK 0 to 4, n=9 TBPEN and 21 CTR) were analysed separately. Effects of TBPEN (vs CTR), bodyweight and RELWEEK were analysed using a linear mixed model with RELWEEK as repeated and pen as random effect.
Results: PRE-ITB the number of predicted feeder visits was lower in TBPEN as compared to CTR and decreased with age (PRED = -18 to -39% at RELWEEK -10 to 0; TBPEN effect p=0.02), leading to a tendency for a shorter daily time in the feeder (TBPEN effect p=0.06). TBPEN showed a growth dip to a -11% PRED level in RELWEEK -9 (TBPEN x RELWEEK p=0.001).
Feeding behaviour changed in TBPEN in RELWEEK -2 to 0. Significant TBPEN x RELWEEK –interactions (p≤0.02) indicated that the relative decrease in the number of feeding bouts accelerated. Together with a progressive shortening of the average feeding bout this led to decreasing relative feed intake and growth (PRED= -10%, -7% and -8% at RELWEEK 0, respectively).
POST-ITB TBPEN still spent less time in the feeder than CTR (TBPEN p=0.04), whereas the difference in the number of visits was decreasing (TBPEN x RELWEEK p<0.001). There was a tendency for a higher intake per second (TBPEN p=0.08) and a significantly faster RELWEEK-related increase in intake per visit (TBPEN x RELWEEK p<0.05), as well as increasingly faster growth (PRED= +9% at RELWEEK 4, TBPEN p=0.02) in TBPEN as compared to CTR. The amount of feed consumed did not differ.
Conclusion: Changes in feeding behaviour in TBPEN 10 weeks before ITB suggests presence of some tail-biting related factor. A growth dip 9 weeks before ITB may indicate the involvement of health problems in tail biting. Rapid changes in feeding behaviour suggest that tail biting behaviour begins or escalates 2 weeks before the first tail wounds are detected. TBPEN shows compensatory growth unrelated to feed intake in the month after ITB onset.

Disclosure of Interest: None Declared

Keywords: feeding behaviour, growth performance, tail biting

Welfare and Nutrition

PO-PT2-111

Comparison of gestating sows housed either in group with the electronic sow feeding system or conventional stall over three consecutive parities

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Introduction: The EU has prohibited the use of gestating crates for pregnant sows. However, keeping pregnant sows in an individual crate is still widely used in Asian countries. As modern type of group housed sows with individual feeding, electronic sow feeding (ESF) system is introduced in EU. However, a few studies were conducted to figure out the long-term and carry-over effects of static group during gestation to lactation on reproductive performance and physical activities of sows. Therefore, this experiment was conducted to compare productivity and physiological status of gestating sows between ESF and conventional stall system up to the third parity.
Materials and Methods: A total of 83 pregnant gilts (Yorkshire×Landrace) were introduced to their treatments, conventional stall (ST) or groups with the ESF system (ESF), on the basis of body weight (BW) and backfat thickness (BFT) in a completely randomized design. Rice hull was used as bedding material in group housing floor for ESF treatment. Commercial gestating diet was provided daily at 2.0 kg, 2.2 kg and 2.4 kg/day in the first, second and third parity, respectively. Sow traits included BW and BFT were measured at d 35, 110 of gestation as well as at 12 h and d 21 postpartum. Reproductive performance, delivery time, weaning to estrus interval (WEI) were measured of sows. For health descriptors, skin injuries and locomotion scores were assessed during gestation.
Results: Backfat gain during gestation tended to be higher in ESF than ST treatment (P=0.08, P= 0.10 in parities 1 and 2, respectively). Similarly, ESF treatment tended to have a more body weight (BW) gain in parities 2 and 3 (P=0.07, P=0.10, respectively). There was as tendency to shorten delivery time in ESF treatment (P=0.08 in parities 1). Higher incidence of body scratch was scored in ESF in early gestation in all parities (P<0.01), resulting in higher locomotor disorders in middle and late gestating period (P=0.07). Higher farrowing rate of sows was observed in ST treatment regardless of parity.
Conclusion: This experiment suggested that ESF treatment showed higher growth performance as well as survival rate of piglets. However, more incidences of body scratch as well as higher locomotion disorder scores were observed in ESF sows due to a combination of persistent fighting around ESF machines and inadequate bedding materials. Consequently it is necessary to consider the proper bedding materials as well as adequate space divider or barrier for gestating sows to avoid escaping aggression in ESF system.

Disclosure of Interest: None Declared

Keywords: ESF system, group housing, sow

Poster Abstracts

Welfare and Nutrition

PO-PT2-113

Assessment Of Animal Welfare And Acid-Base Imbalance In Pigs That Recovered Consciousness After Co2 Stunning.

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Introduction: During slaughtering procedure, every animal should be unconscious chiefly to avoid inflicting undue pain during bleeding. Exsanguination without prior stunning is an extremely controversial practice from the standpoint of animal welfare because some animals take a long time to lose brain function. CO_2 stunning can be reversible or irreversible; pigs may recover consciousness before dying, such that the time between stunning and exsanguination is a determining factor for the efficiency of the stunning process. Some researchers have described that pigs should be exposed rapidly to 90% CO_2; though it has been shown that exposure to CO_2 at 90% for 120 s is more effective than exposure for 90 s in abolishing consciousness reflexes. The objective of the present study was to evaluate the effect of different concentrations of CO_2 on the stunning of pigs by measuring physiometabolic blood profiles.
Materials and Methods: A total of 1336 pigs were stunned in a CO_2 chamber for approximately 90 s. The pigs were classified into four groups: the first group rested in pens and was sampled 3 h before slaughtering (reference values, RV). The rest of the pigs were assigned to 3 groups according to the CO_2 concentration used for stunning: 85, 90 or 95%. Each group was then further divided into 2 sub-groups: (*A*) pigs exsanguinated during the first 60 s after leaving the chamber without recovering consciousness (WRC), and (*B*) pigs that were exsanguinated after more than 60 s and did recover consciousness (RC).
Results: Blood pH of the RC pigs was below 7.08, while blood Ca^{2+} (>1.59 mmol/L), glucose (>159.79 mg/dL) and lactate (>103.52 mg/dL) levels all increased compared to the control group ($P<0.05$). All pigs exposed to CO_2, regardless of concentration, presented changes in critical blood variables, and exposure to the gas also affected their acid-base balance, producing a process of acidosis, hyperglycemia, hyperlactatemia, hypercapnia and hyperpotasemia. This physiological disequilibrium was greater when the animals recovered consciousness seconds after leaving the stunning chamber.
Conclusion: Therefore, it is necessary to reduce waiting times between removals of the pigs from the stunning chamber and performing exsanguination. Under no circumstances should this interval exceed 60 s, otherwise the pigs may recover sensitivity. The study recommends maintaining strict control of entry into, and removal from, the CO_2 chamber in order to avoid backlogs in the slaughtering area and so ensure that the pigs do not regain consciousness.

Disclosure of Interest: None Declared

Keywords: CO2 stunning, acidosis, consciousness

Welfare and Nutrition

PO-PT2-114

Assessment Of Animal Welfare And Behavioral Changes In Pigs During Co2 Stunning Procedure.

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Introduction: Early CO_2 stunning of pigs is associated with a process of aversion and stress, characterized by different induce behavioral changes. The effects of CO_2 act in two ways: first, causing irritation membranes of the nasal mucosa; and second, as an agent that strongly stimulates the respiratory center, causing hyperventilation and suffocation before losing consciousness. However, signs of aversion as 'retreat attempts' or 'escape' have been reported in pigs just before completing the state of anesthesia; although, the record of behavioral changes during stunning has been a useful tool for determining the absence of welfare in pigs. The observation and quantification of these behavioral changes and design ethograms during stunning, give guidelines to understanding the process of pig aversion and stress when exposed to CO_2. This fact indicates that just before the pig lose consciousness, there is a transition phase, in which a number of behavioral changes can be observed, such as: 'breathing efforts' with the open mouth, 'ataxia' and 'attempts to incorporate'. The objective of the present study was to evaluate the effect of CO_2 stunning exposure time on pigs by measuring several behavioral changes.
Materials and Methods: A total 336 pigs were videotaped during the first 30 s of the stunning process (at 86% CO_2) and then classified in groups according to the time they were exposed to the gas, as follows: T_1= 0-to-10 s, T_2= 11-to-20 s, and T_3= 21-to-30 s. Sixteen different behavioral traits were recorded and analyzed according to frequency percentage, prevalence, and duration (s).
Results: In T_1, the most frequent and prevalent behaviors were sitting and standing (assimilation phase); in T_2, pigs attempting to escape and stand up showed higher frequency and prevalence percentages (excitation phase); while in T_3, the most common behavioral indicators were difficulty in maintaining posture and muscular contractions (anesthetic phase). 'Attempts to escape' and 'loss of posture', have been the most reported behavioral indicators around the world, these denote a clear lack of welfare during stunning in pigs.
Conclusion: CO_2 stunning method is not conducive to pig welfare in slaughter plants, due to the different behavioral changes that pigs recorded, which are regarded as behaviors of aversion. However, the CO_2 concentration used in this study was effective to stun pigs, but causes physical stress and may devalue the last minutes of life of pigs before exsanguinated.

Disclosure of Interest: None Declared

Keywords: CO2 stunning, aversion, etology.

Welfare and Nutrition

PO-PT2-115

Effects of lysolecithin supplementation on performance and metabolic parameters in sows

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Introduction: Emulsifiers containing lysolecithin and phospholipids improved nutrient digestibility in growing pig diets in earlier work. To date, lysolecithin supplementation has not been evaluated in diets of gestating and lactating sows. The objective of the present study was to evaluate if lysolecithin could enhance sow performance through increased nutrient availability. A study was set up with supplementation of lysolecithin during the last three weeks of the gestation period and throughout the lactation period of sows, measuring sow performance, selected metabolites and energy balance through plasma leptin.
Materials and Methods: In total 60 sows (TOPIGS40) were allocated to two treatments: a) CON (control group): the sows received standard diets during late gestation and lactation; b) LYS (lysolecithin supplemented group): the sows received respective rations supplemented with 750 mg/kg feed of a commercial product containing 50% lysolecithin (Lysoforte Booster Dry™). The study started at day 90 of gestation, and was completed at day 28 of lactation. All relevant parameters for sow and litter performance were recorded. Moreover at day 108 of gestation and at day 14 of lactation the sows were subjected to blood sampling. The blood parameters investigated, were related to energy and lipid metabolism (glucose, leptin, NEFA, urea, creatinine, triglycerides).
Results: Backfat thickness was lower in LYS group compared to CON at day 108 of gestation and at weaning (P=0.030 and 0.044 respectively), yet with no difference in the mm of the backfat tissue mobilized during lactation. Litter weight at weaning was higher in the LYS than CON (P=0.027). Leptin levels were lower in the LYS than CON at 108 day of gestation (P=0.015). Fasted glucose levels at day 14 of lactation tended to be lower in the LYS compared to CON (P=0.074). Urea levels were higher in the LYS than CON at day 14 of lactation (P=0.002), whereas creatinine remained unaffected.
Conclusion: The LYS sows arrived 1 week prior farrowing with less back fat reserves, which agreed with the lower leptin concentrations. Yet, they were better in supporting litter growth, as reflected in the higher litter weight at weaning. The lower glucose levels of LYS sows at fasted state at day 14, indicate a better peripheral blood glucose utilization. The higher postprandial urea levels, may indicate a more efficient dietary nitrogen absorption in the LYS sows during lactation, since creatinine was unaltered. Further study needs to clarify if lysolecithin supplementation exerts this effect on performance and metabolism through late-gestational changes in nutrient availability or other mechanisms.

Disclosure of Interest: G. Papadopoulos: None Declared, M. Di Benedetto Conflict with: Participation in study design, G. Janssens: None Declared, G. Arsenos: None Declared, P. Fortomaris: None Declared

Keywords: lysolecithin, metabolism, sows

Welfare and Nutrition

PO-PT2-116

Is there an impact of diet's physical form on stomach barrier function (in vivo / in vitro) against artificially aplied E. coli (F4, STI, STII, LTI)?

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Introduction: It is well known that stomach barrier (acidity) acts against diverse pathogenic agents. This function is particularly influenced by particle size distribution in the diet. Diarrhoea causing *E. coli* is an agent of relevance in today's pig production that can lead to weight gains up to high losses. An experimental study was conducted to evaluate the ability of a specific pathogenic *E. coli* to survive stomach passage, in vivo and in vitro (exposed to stomach content).
Materials and Methods: A coarsely ground meal diet (CM) and a finely ground pelleted diet (FP) being botanically and chemically identical (13.8 MJ ME, XP: 237, XF: 42 g/kg as fed) were each fed ad libitum to 9 weaned barrows housed individually. After 5 weeks the piglets were divided in two groups, 4 piglets out of each feeding group were infected artificially with an oral dose of 1.4 x 10^9 cfu *E. coli* (F4, STI, STII, LTI) and euthanized two hours later to count the applied *E. coli* in content of stomach and small intestine. Remaining 5 piglets were euthanized and samples of stomach content were taken quartered. From these samples 10g were inoculated in vitro with 1.3 x 10^9 cfu of the *E. coli* mentioned above and the counts of the agent were determined after 3, 60, 120 and 240 min of exposition in a shaking water bath (37°C). For quantitative analysis serial dilution and growing on *Columbia Agar* with 5% sheep blood (37°C, 24h) were used. In stomach content DM content and pH value were determined additionally. Statistical analysis was performed by procedure mixed (SAS Enterprise guide 5.1), $p<0.05$.
Results: After artificial infection the concentration of applied *E. coli* in the cranial small intestine differed significantly (CM: 5.40, FP: 6.94 cfu/g) but no difference was seen in stomach content. In vitro 86 to 91% of the inoculated *E. coli* dose was counted with no difference between the groups and the time of incubation. Stomach content was firm and layered in pigs fed CM (in the four quarters: DM: 34.4, 31.9, 27.2, 26.2%), these piglets had significantly differing pH values (pH: 4.94, 4.76, 3.74, 3.86). Piglets fed FP showed significantly lower DM content in a fluid stomach content (20.1, 21.7, 23.8, 20.1%) and no pH gradients (pH: 4.52, 4.45, 4.44, 4.41).
Conclusion: As known from earlier studies grinding intensity had a great impact on the quality of stomach content and with this on stomach barrier function. But the applied *E. coli* survives when exposed to the stomach content (in vivo, in vitro) independent of the different conditions due to the physical form of the diet so that contrary to other pathogenic agents (e.g. Salmonella) stomach barrier seems to have less impact on the defence against this distinct *E. coli*.

Disclosure of Interest: None Declared

Keywords: Escherichia coli, grinding intensity, stomach barrier function

Poster Abstracts

Welfare and Nutrition

PO-PT2-117

Different Levels of Palm Kernel Meal in Weaning Pigs and Carry-over Effect on Marketing Pigs in Growth and Pork Quality

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Introduction: The fluctuation in price of major feed ingredients is affecting cost of production in swine industry. Therefore, alternative ingredients to reduce feed cost become more important than before. Palm kernel meal (PKM) is a by-product after extracting palm oil in tropical area. There were various studies to evaluate PKM supplementation instead of corn and SBM in growing-finishing pig's diet. However, there was no information for optimal level of dietary PKM in weaning pigs' diet, this experiment was conducted to evaluate the effects of different levels of PKM in weaning pig diet on growth performance, blood profiles, nutrient digestibility and pork quality.
Materials and Methods: A total of 350 weaning pigs were allotted to one of 5 dietary treatments based on body weight (BW) and sex in 7 replicates with 10 pigs per pen using a randomized completely block (RCB) design. After weaning periods, 245 selected pigs by BW of each treatment and sex were moved to growing-finishing facility to evaluate carry-over effect on growth performance and pork quality. Dietary treatments were divided by different levels of PKM (0, 4, 8, 12, or 16%) during weaning periods. In growing-finishing periods, all pigs were provided the same commercial feed which consisted of 4 phase feedings. In addition, blood profiles, nutrient digestibility and pork quality were evaluated. Data were analyzed by analysis of variance using the general linear model procedure of SAS.
Results: When weaning pigs were fed at 16% of PKM, lower BW, ADG and ADFI were observed than those of other treatment ($P<0.01$). Although different growth responses were observed by dietary PKM during weaning period, there were no significant differences among treatments in growing-finishing period. In blood profiles, no difference was observed on BUN, IgA and IgG concentration during the whole experimental period. Crude protein digestibility of weaning pigs fed 4% PKM diet was significantly higher than other treatments ($P<0.05$). In pork quality, pH, L* and a* value were not affected by supplementation level of PKM in diet of weaning pigs. However, b* value at 0 hour was significantly lower when pigs were fed diet containing 16% of PKM ($P<0.05$). Water holding capacity and cooking loss were increased linealy as increasing PKM level in weaning pig diet ($P<0.01$).
Conclusion: This experiment suggested that supplementation of 16% of PKM in weaning pig diet showed negative effect on growth performance. However, dietary PKM can be supplemented up to 12% in weaning pigs' diet without detrimental effects on growth and pork quality at market weight.

Disclosure of Interest: None Declared

Keywords: Digestibility, Palm kernel meal, Pork quality

Welfare and Nutrition

PO-PT2-121

Farrowing culling and therapeutic injections rates in litters from sows treated with Metacam

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Introduction: Farrowing is a critical period for the sow in which painful, inflammatory and infectious processes along with other systemic events cause post-partum stress and may trigger Postpartum Dysgalactia Syndrome (PPDS) which manifests itself either in clinical or subclinical presentation, both with negative impact on the sow and its litter regarding the development of suckling pigs. The objective of this study was to determine under field conditions the effect of meloxicam in litters from sows of a farm with subclinical PPDS.
Materials and Methods: The study took place on a multi-site intensive farm with a total number of 265 sows and 3077 piglets. Sows were divided into 2 experimental groups, Group A (n=127 sows) was treated with meloxicam 5 ml IM and Group B (n=138 sows) was injected with saline solution 5 ml IM to serve as control group. One hour after farrowing each piglet was identified with numbered ear tags and cross fostering was restricted to first 24 hours within litters from the same treatment group. The number of culled animals was documented alongside the number of therapeutic injectable medications on an individual level. The experimental unit was the litter, chi-square test was used to assess differences of culled animals and a significance test for independent proportions was used to assess the number of therapeutic injectable medications between groups.
Results: Piglets in the control group had a greater culling rate than in the meloxicam group. Control group had 34 culls out of 1573 animals and meloxicam group had 18 culls out of a total of 1504 animals. This difference was statistically significant ($p<0,05$).
Overall, the number of therapeutic injections applied in relation to the total number of piglets per group was 20% for the meloxicam group and 25% for the control group. 43 concomitant therapeutic injections where given in the meloxicam group to control locomotory problems, versus 45 injections in the control group. In terms of diarrhea, 261 therapeutic injection where given in the meloxicam group versus 348 injections in the control group, this difference was statistically significant ($p< 0,05$).
Conclusion: The litters from sows treated with meloxicam performed better in culling rate and therapeutic injections rate from birth to weaning (21 days average). The differences found on both variables could be related to a sow with less pain and inflammation after farrow resulting in a greater availability of milk and colostrum for the piglets during the critical starting process of lactation. These results are in line with previous research and further confirm the value of controlling lactation disorders during the first critical hours for piglets colostrum and milk intake.

Disclosure of Interest: C. Gutiérrez Conflict with: Boehringer Ingelheim, D. Guiñez: None Declared, V. Grosse-Liesner: None Declared, A. Ruiz: None Declared, C. Roudergue Conflict with: Boehringer Ingelheim

Keywords: Meloxicam, Metacam, NSAID

Welfare and Nutrition

PO-PT2-123

Associations between carcass tail lesions and other welfare conditions and the performance of negative behaviours in pigs

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Introduction: Tail lesions are outcomes of tail biting behaviour and reflective of impaired welfare in pigs. It is possible that tail biting is associated with other behavioural problems such as ear- or flank biting. Hence, the aim of this study was to investigate the potential of carcass tail lesions to reflect other behavioural problems on farms and thereby act as an 'iceberg indicator' for pig welfare.
Materials and Methods: Welfare assessments were conducted on 31 integrated pig farms by observing 18 randomly selected pens of 1st and 2nd stage weaner and finisher pigs (6 pens per stage). Pens were observed for 10 min and the number of pigs with tail, ear, flank and skin lesions was recorded. All occurrence behaviour sampling was used to record frequency of tail-, ear-, and flank biting (5 min). The average percentage of pigs in a pen affected by welfare lesions was calculated, as was the average frequency of behaviours per pig, in each production stage (mean ± SE). One batch of pigs from each farm was observed at the slaughterline and carcass tail lesions were scored according to severity (0 – 4). An average carcass tail lesion score for each batch was calculated. Spearman rank correlations were calculated between average carcass tail lesion score and the measured welfare conditions and behaviours.
Results: All lesion types were observed in the 1st stage pens (tail: 5.4 ± 1.0%; ear: 9.4 ± 1.7%; flank: 0.4 ± 0.4%; skin: 4.8 ± 0.7% of pigs affected). The percentage of pigs with tail lesions in 2nd stage weaner pens was 6.2 ± 0.5% with the highest prevalence recorded in finisher stage pens (11.0 ± 0.8%). Similarly, flank lesions were more highly prevalent in 2nd stage weaner and finisher pens (1.4 ± 0.5% and 2.3 ± 0.5%, respectively). Ear lesions were most prevalent in 2nd stage weaner pens (15.4 ± 2.8%) with the lowest prevalence seen in the finisher pens (7.1 ± 1.3%). On average 204.3 ± 25.8 carcasses were scored per batch and average carcass tail lesion score was 0.83 ± 0.02 (range: 0.53 – 1.26). No correlations were found between carcass tail lesion score and the prevalence of tail/ear/flank lesions or frequency of tail/ear/flank biting behaviour for any of the production stages.
Conclusion: The high prevalence of ear and tail lesions throughout the production stages raises concern for pig welfare. No associations were found between carcass tail lesion scores and the abnormal biting behaviours or consequent lesions, suggesting that this measure might not be a useful 'iceberg indicator' for these aspects of pig welfare.

Disclosure of Interest: None Declared

Keywords: Abnormal behaviour, Carcass tail lesions, Welfare lesions

Welfare and Nutrition

PO-PT2-124

Ultraviolet treated liquid porcine plasma and spray dried to powder form maintained product quality

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Introduction: Spray dried plasma (SDP) is a feed ingredient included in pig starter diets. Numerous scientific papers and extensive review papers document significant improvement in post weaning performance and its safety when SDP is included in weaning feed. Ultra violet light at 254 nm (UV-C) kills many microorganisms including bacteria and viruses. Treatment of human plasma fractions with UV-C has been effective in inactivation of many model viruses and both Gram negative and Gram positive bacteria. The objective was to evaluate the effect of a unique UV-C reaction system designed for processing opaque liquids in the production of porcine SDP and its impact on total aerobic plate count (TPC) and typical quality parameters.
Materials and Methods: Two commercial production runs were completed. The first run included the processing of 3 truckloads (~22,700 L each) porcine plasma collected at different commercial abattoirs on 3 different days. The liquid plasma was treated with UV-C at a rate of 2,836J/L, concentrated and spray dried. This would represent a partial-day production. The second run comprised processing 9 truckloads of porcine plasma obtained from different collection sites representing a typical full-day production. The porcine plasma was UV-C treated with 2,853J/L, concentrated and spray-dried. Plasma for both runs met all product quality specifications for commercial plasma and with the exception of UV-C treatment was processed consistent with normal production SOP's. Samples were analyzed for viral genome by PCR (PEDV, PCV2, PRRS, PPV, and Deltacoronavirus), TPC, pH, protein, solids, ash, and porcine IgG. Samples were submitted to a commercial lab for TPC, PCR analysis was conducted at Iowa State Diagnostic Lab, while other analysis was conducted by the Quality Assurance lab at APC. The Reynolds number was determined to assure turbulent flow was maintained through the UV-C reactor. Analysis of the UV-C treated SDP was compared to typical values for commercially produced SDP.
Results: UV-C treatment reduced TPC 3 log in liquid plasma and more than 2 log reduction compared to untreated commercial powder plasma. UV-C treated plasma met all specifications for commercial plasma. UV-C treatment did not affect PCR analysis for the viruses that were evaluated.
Conclusion: These tests demonstrate that UV-C treatment of liquid plasma effectively reduces TPC. Further, UV-C treatment of liquid plasma does not affect other quality parameters of SDP. The addition of UV-C treatment is an effective redundant safety step in the commercial production of SDP.

Disclosure of Interest: J. Campbell Conflict with: Employee, J. Polo Conflict with: Employee

Keywords: safety, spray dried plasma, ultraviolet light

Poster Abstracts

Welfare and Nutrition

PO-PT2-133

Automated rating of welfare indicators for pigs in the slaughterhouse – a pilot study

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Introduction: In Germany farmers are legally obligated to monitor animal welfare indicators, and based on that, improve their conditions. Besides the difficulties in defining indicators, the comparable assessment is another challenge. Therefore in the poultry industry, camera systems have been installed in slaughterhouses to evaluate the foot pad condition with a software. The results are open to farmers as feedback and partly to veterinary departments. Such a system has not been built up in pig slaughterhouses yet, mainly due to the more complex anatomy and the lack of suitable defined indicators. Therefore, the aim of this study is to develop a commercial camera system for the assessment of animal welfare indicators for pigs in the slaughterhouse.
Materials and Methods: In this pilot study the focus was on external indicators, which can be recorded directly in the slaughterhouse with a camera system (CLK GmbH, Münster, Germany). The system was installed in a German slaughterhouse slaughtering 3,500 – 5,000 pigs per day. It was located after the cleaning of the pigs. The system included five cameras and a special one for the 3D pictures, lights, triggers, a blue background. It took eight pictures of every pig (head, back, hind quarter, both lateral views, three 3D pictures of the joints of the hind legs).
These pictures were interpreted by a veterinarian, discussed in a consortium, and used by the manufacturer to develop a program for an automated evaluation. In the next step, the automated results are controlled by an individual observer.
Results: Ear necrosis, tail damages, wounds from chasing and tattooing, and swellings of the joints of front and hind limbs were defined as indicators in this project. These indicators are in agreement with the German Quality Assurance Initiative (QS GmbH, Bonn, Germany).
Between the 11-09-15 and 05-01-16 approximately 750,000 pictures were taken. Until June, 2016 presumably 4,400,000 new pictures will be taken. These pictures are analysed and categorized critically.
Hundreds of pictures of indicators and differential diagnosis were found yet. Based on these, a software is currently developed. In cooperation of veterinarians and the manufacturer, the pictures were optimized. The correct classification of every pig was guaranteed. Moreover, pictures were taken manually in the stables to compare the results of the camera and the original condition.
Conclusion: First steps for an automated rating system of animal welfare indicators for pigs in the slaughterhouse have been made. Resulting in a powerful tool to support the objective monitoring of animal welfare, also in large animal numbers, it will lead to a better, comparable feedback both to the farmer and the veterinarian.

Disclosure of Interest: None Declared

Keywords: Animal Welfare, Slaughtering, Quality Control

Welfare and Nutrition

PO-PT2-139

Effects of Alkaloid Extracts on Growth Response and Gut Histology of Nursery Pigs

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Introduction: Weaning process causes a significant biological stress to piglets which lead to a detrimental effect on feed intake, growth rate, intestinal morphology and immune system. A mixture of quaternary benzophenanthridine alkaloids and protopine alkaloids (QBA+PA) possess antimicrobial and anti-inflammatory effects. They also are known to improve endogenous digestive enzyme secretion, activate of the immune system and improve protein retention of farm animals.
Materials and Methods: A total of 240 three-way crossbred pigs (LW x LR x D) at 4 weeks of age were used to evaluate the effects of QBA+PA mixture on growth response and gut histology of nursery pigs. A completely randomized (CRD) design was used for this study. All piglets were randomly divided into 4 dietary treatments including, control diet and control diet supplemented with QBA+PA mixture at 100, 150 and 200 ppm, respectively. Each treatment consisted of 6 replications with 10 pigs per replication (5 castrates and 5 females). Feed and water were provided *Ad*-libitum throughout the experimental period (4-9 weeks of age).
Results: During 4-7 weeks of age, the supplementation of QBA+PA mixture significantly increased ADG of nursery pig ($P<0.05$) when compared to that of the control group. The supplementation of QBA+PA mixture at the levels 100 and 150 ppm significantly improved FCR ($P<0.05$). During 7-9 weeks of age, the supplement of QBA+PA mixture significantly increased FI, ADFI, ADG ($P<0.05$) and improved FCR of nursery pig ($P<0.05$). The results showed that QBA+PA mixture supplementation significantly increased piglet performance during 4 to 9 weeks of age. However, QBA+PA mixture supplementation had no effect on gut histology.
Conclusion: It could be concluded that the supplementation of QBA+PA mixture improves growth performance of nursery pig.

Disclosure of Interest: None Declared

Keywords: alkaloid extract, nursery pigs, growth performance

Welfare and Nutrition

PO-PT2-141

Efficacy of dietary ethyl-enzyme liquid energy on growth performance, nutrient digestibility, meat quality-carcass grades of grower-finisher pigs

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Introduction: Previous studies have led to observations regarding the negative impact of non-starch polysaccharides in corn-soybean meal diets on nutrient digestibility. Therefore, steps are undertaken to positively influence nutrition in livestock by utilizing enzymes. This experiment was conducted to evaluate the effects of ethyl-enzyme liquid energy (ELE) on growth performance, nutrient digestibility, meat quality traits and carcass grades of grower-finisher pigs.
Materials and Methods: In this study, a total of 180 pigs [(Landrace×Yorkshire)×Duroc] with initial weight of 23.3±1.40 kg were used for 16 week (wk) feeding trial. Pigs were randomly allocated to 3 treatments [T1-CON (Basal diet), T2 (-50kcal/kg of CON diet), T3 (T2+1% Alcopro®)] according to their sex and body weight (BW) as 12 replicates/treatments, with 5 pigs/pen. Ethyl-enzyme liquid energy (Alcopro®, Simco Nutrition Group™, USA) is a commercially available ethanol based liquid feed additive containing energy source (about 10,000 kcal/kg ME) and natural digestive enzymes for livestock. It is not oxidized and rancid, a stable liquid in storage. Pigs were weighed at initial, wk 2, 6, 8, 12 and 16 of the experimental period while feed consumption was recorded on a per pen basis to calculate the growth performance traits and to test the effect of liquid energy supplementation (T1vs.T2; T1vs.T3; T2vs.T3) using orthogonal contrasts. Significance was defined as P≤0.05.
Results: Our results showed significant (P≤0.05) effects on BW, growth performance of average daily gain, average daily feed intake (ADFI) and gain:feed (G:F), no (P>0.05) effect on G:F at grower stage and ADFI at finisher stage was observed. Supplementation of ELE showed significant effects (P≤0.05) on nutrient digestibility of energy (E) at wk 6 and dry matter (DM) at wk 16; live back fat thickness (BFT) at wk 12 and 16. Our results depict significant effects (P≤0.05) on meat color (L*), carcass weight and BFT. We also observed equal number of "1+" carcass grade in pigs fed with basal diet and ELE supplemented diet and higher number of "1" carcass grade in pigs fed with ELE supplemented diet than others.
Conclusion: In conclusion, these results indicate optimistic effects of ELE supplementation in corn-based soy bean meal on performance of pigs and potentially it may increase the dietary energy level economically. Although this study only provides a limited report on ELE supplementation, further research is necessary to determine if an energy response from ELE can be found in grower-finisher pigs.

Disclosure of Interest: None Declared

Keywords: Corn-soybean meal, Ethyl-enzyme liquid energy, Grower-finisher pigs

Welfare and Nutrition

PO-PT2-144

Effects of supplementing growing-finishing pig diets with Bacillus spp. probiotic on growth performance and meat-carcass grade quality traits

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Introduction: Using antibiotics as growth promoters in animal feeds has been forbidden since 2011 in South Korea. Probiotic have received considerable attention as suitable alternatives of antibiotics to promote growth in the pig industry. Among several bacterial species used as probiotic, spore forming *Bacillus* spp. has been considered as the most appropriate probiotic as its spores can to resist harsh environments, thus enabling extensive storage at ambient temperature. However, reports on feeding a combination of *Bacillus* spp. probiotic to growing-finishing pigs are rare.
Materials and Methods: A total of 75 pigs [(Landrace×Yorkshire)×Duroc] with an initial body weight (BW) of 23.3±1.40 kg were used to investigate the influence of dietary *Bacillus* spp. probiotic (*B. coagulance* ($1x10^9$ cfu/g), *B. lichenformis* ($5x10^8$ cfu/g) and *B. subtilis* ($1x10^9$ cfu/g) in growing-finishing pig on performance parameters with a 16 weeks (wk) feeding trial. Pigs were randomly allocated to 3 treatments [T1-CON (Basal diet); T2 (CON+0.01% probiotic); T3 (CON+0.02% probiotic)] according to their sex and BW as 5 replicates/treatments, with 5 pigs/pen. These dietary treatments were given as Phase I (grower, 0-6 weeks) and Phase II (finisher, 6-16 weeks) to analyze the growth performance traits at the start and at weeks 6, 12, and 16 of the experimental period. Orthogonal polynomial contrast was conducted to measure the linear and quadratic effects for increasing the *Bacillus* spp. probiotic levels on all measurements.
Results: The entire experiment using dietary probiotic revealed significant effects on average daily gain and gain:feed were observed, but no effects on average daily feed intake. The result showed significant effects on digestibility of dry matter (P=0.002), nitrogen (P=0.069), and energy (P=0.099) at wk 16; number of fecal *Lactobacillus* (P=0.082; 0.041), *E. coli* (P=0.097; 0.052) and blood glucose (P=0.001; 0.049) at wk 6 and 16. Dietary supplementation with *Bacillus* spp. probiotic resulted in linear significant effect on sensory evaluation of meat color (P=0.025), drip loss at d 3 (P=0.013), and carcass weight (P=0.034) in pigs. However, no significant effects on blood metabolic profiles, noxious gas emissions in this experiment.
Conclusion: Dietary combination of *Bacillus* spp. can be used as probiotic for enhancing the growth performances and carcass quality in growing-finishing pigs. Nevertheless, using *Bacillus* spp. based complex probiotic to improve meat quality has been questioned because the results in pigs have been inconsistent. Further research should be conducted to determine the impact dietary *Bacillus* spp. probiotic may have on meat quality traits.

Disclosure of Interest: None Declared

Keywords: Bacillus spp., Growing-finishing pigs, Probiotic

Poster Abstracts

Welfare and Nutrition

PO-PT2-145

Farrowing time in sows is reduced by an herbal extract, containg the active form of vitamin D3

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Introduction: Modern pig farming is still associated with a high pre-weaning mortality, which is partly caused by a slow progress of piglet expulsion. Most of the deaths occur around the time of farrowing and therefore farrowing is the most critical phase in pig production. The impact of farrowing includes not only the litter survival and subsequent health of the piglets, but also the feed intake and fertility of the sows. So farrowing rate is a main factor for consistent pig production and profitability of pig farming. Since tedious labor may be caused by weak muscle tonus, dietary calcium levels are expected to be important. Calcium homeostasis, is regulated by vitamin D and its active metabolites.
Solanum glaucophyllum, a South American plant, cultivated for its content of $1,25(OH)_2 D_3$, contains the active form of vitamin D in a glycosidic form. The water-soluble extract of the plant is converted into a standardized granulated powder, characterized as “Solbone”, containing 50 microgram $1,25(OH)_2 D_3$ per g. This active metabolite of vitamin D is able to improve calcium homeostasis in chicken, cows and in pigs. Our hypothesis was, that “Solbone” influences the farrowing time positively and as a consequence the survival and health of piglets as well as the fertility of the sow might improve.
Materials and Methods: In an organic piglet producing farm, seven sows (German Large White x German Landrace) were mated with a Pietrain boar. One week before calculated parturition date, the animals were moved into individual farrowing pens with video cameras installed. Solbone application via feed (1g per day and per sow) started seven days before the expected and calculated parturition. The animals were randomly assigned to a control group or Solbone group. In the second trial (6 months later) a cross-over trial was performed. So over a total of four trials each animal was assigned twice to the control group and twice to the Solbone group. Calcium and $1,25(OH)_2 D_3$ were measured in blood before and after supplementing “Solbone” for one week.
Results: Serum $1,25(OH)_2 D_3$ increased significantly under the influence of “Solbone”. Blood calcium was higher in the “Solbone”-fed sows as those in control groups. As outcome over four experimental rounds a significant reduction of 25 % (from 279 minutes to 209 minutes) in farrowing time was observed under the vitamin D product as compared to control.
Conclusion: A reduction of the farrowing time in sows might reduce pre-weaning mortality, since it is well-known that stillborn piglets are delivered after longer birth intervals than liveborn piglets.

Disclosure of Interest: None Declared

Keywords: Farrowing time, Solanum glaucophyllum, Vitamin D

Welfare and Nutrition

PO-PT2-148

Forensic Cases of Ulcerations in Pigs

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Introduction: Ulcerations in the skin of a pig may be painful and hamper the welfare of the animal if neglected. Serious and untreated ulcerations are reported to the police by veterinary enforcement officers, and often an examination carried out by a veterinary pathologist is requested. A retrospective study of forensic case files concerning ulcerations in Danish pigs from 2000 to 2014 was carried out. The aim of the study was to evaluate the number of cases, the number of pigs involved, the anatomical localization of ulcerations and the changes during the years.
Materials and Methods: Case files regarding ulcerations submitted for forensic examination from 2000 to 2014 were evaluated retrospectively. In the files, the following information was analysed: the age and the sex of the animals and the size and the anatomical localization of ulcerations.
Results: In total, 209 case files concerning 283 pigs or parts of these with 459 ulcerations were included. Despite a decline in cases since 2004 the number of pigs remained fairly constant. The case files included between 1 and 10 pigs with an average (X) ± standard deviation (SD) of 1.4±1.2 pigs and each pig had 1 to 60 ulcerations (1.7 ±3.7, ±SD). The pigs were registered as females (53%), males (5%) and of unknown sex (42%). Approximately half of the pigs were sows (51.2%), while 34.3%, 6.7%, 0.4% and 7.4% were slaughter pigs (5 to 6 months), younger pigs (< 5 months), adult boars and pigs of unknown age, respectively. In 2004, 2005, 2007, 2008, 2009 and 2011, sows with shoulder ulcerations were the most frequent while in 2014 pigs with ulcerations on umbilical outpouchings dominated. Since 2011 the number of sows with shoulder ulcerations has declined. Ulcerations on the body, limbs or tail were less frequent but present throughout the entire study period.
Conclusion: Despite a decline in the number of case files the annual number of pigs remained constant suggesting fewer but more serious offenders. Sows with shoulder ulcerations were the most frequent; however, from 2011 the number declined most likely reflecting an increased focus on this type of lesion in Danish sows during the last 10 years.

Disclosure of Interest: None Declared

Keywords: Forensic, Ulceration, Welfare

Welfare and Nutrition

PO-PT2-149

Relationship between body temperature and systemic inflammation markers induced by lipopolysaccharide in dependence of an oral deoxynivalenol exposure

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Introduction: Body core temperature is a stable parameter under physiological conditions as well as an important cardinal symptom for clinical monitoring in systemic inflammation. Besides fever further indicators of an inflammatory reaction are changes in white blood cell counts, TNF-α and kynurenine-tryptophan ratio, but their interrelationship is not yet fully clarified. Thus we investigated these relationships in healthy as well as in pigs exposed to LPS and/or DON.
Materials and Methods: A total of 44 barrows were exposed for 4 weeks either to a DON-contaminated (4.59mg DON/kg feed) or a control (CON) diet. They were surgically equipped with an intraabdominal temperature logger and a multi-catheter system (*V.portae hepatis, V. lienalis, Vv.jugulares int. et ext.)* to facilitate simultaneous infusion of either 0.9%NaCl (CON) or LPS (7.5µg/kg BW) for 60min and venous blood sampling. Body temperature was measured every 5min and blood samples for leukocyte counts, TNF-α and kynurenine-tryptophan analysis (Kyn-Trp ratio) were taken every 15min, from 30min before until 180min after start of infusion. The combination of diet and infusion created six groups: CON_$CON_{jug.}$-$CON_{por.}$, CON_$CON_{jug.}$-$LPS_{por.}$, CON_$LPS_{jug.}$-$CON_{por.}$, DON_$CON_{jug.}$-$CON_{por.}$, DON_$CON_{jug.}$-$LPS_{por.}$, DON_$LPS_{jug.}$-$CON_{por.}$. Data were evaluated by PROC MIXED with group and time and their interaction and PROC CORR (SAS Enterprise Guide 6.1).
Results: Leukocytes: LPS-induced leukopenia from 15min *p.i.*, with lowest levels at 75min *p.i.* and temperature increased at the same time and reached a plateau at 60min *p.i.*. Sole DON-feeding resulted in higher leukocyte counts (p=0.04). Combined treatment yielded an earlier leukopenia (p<0.05) and lower temperature rise (~0.5°C, p=0.08) in DON_$LPS_{jug.}$-$CON_{por.}$ compared to CON_$LPS_{jug.}$-$CON_{por.}$. A significantly positive correlation between both parameters was found in DON_ $CON_{jug.}$-$CON_{por.}$, while all LPS groups were negatively correlated (p<0.001) with the strongest relationship in CON_$LPS_{jug.}$-$CON_{por.}$ (R=-0.7).
TNF-α: LPS induced a sudden increase in TNF-α from 30min *p.i.*, peaking at 60min *p.i.*. Significantly positive correlations with temperature were found in all LPS groups (p<0.05) with the strongest relationship in CON_$LPS_{jug.}$-$CON_{por.}$ (R=0.4).
Kyn-Trp ratio: LPS induced an increase in ratio at 180min *p.i.* (p<0.001). Significantly positive correlations were found in both DON-LPS groups and, with the strongest relationship, in CON_$LPS_{jug.}$-$CON_{por.}$ (R=0.6).
Conclusion: We were able to confirm a significant relationship between body core temperature and leukocyte counts, Kyn-Trp ratio and TNF-α in descending order under pathophysiological conditions and with the strongest dependency in control-fed, systemic LPS-infused pigs.

Disclosure of Interest: None Declared

Keywords: body temperature, inflammation markers, physiological and pathophysiological conditions

Welfare and Nutrition

PO-PT2-150

Molecular characterisation of idiopathic lumbar kyphosis in pigs

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Introduction: A humpy-backed syndrome of pigs has persisted in the British pork industry and causes of the deformity have been difficult to identify (Penny RHC, 1986). The disease presents challenges in regards to handling the carcass (Holl et al , 2008) and is suspected to slow down growth rate (Straw, Bates, & May, 2009). There is no clear evidence of the biological mechanisms by which kyphosis is induced. Through collecting tissue samples from affected and healthy pigs over 3 age groups, this study aimed to identify molecular mechanisms induced by kyphosis.
Materials and Methods: A range of tissue samples such as serum, liver, kidney, small intestine, bone and vertebral cartilage were dissected from kyphotic and control pigs at pre-weaning (2 weeks), weaning (5 weeks) and post-weaning (13 weeks). RNA was extracted from tissue samples using the RNeasy lipid tissue mini-kit (Qiagen) and cDNA was synthesised using the Transcriptor first strand cDNA synthesis kit (Roche). Expression levels of *BMP7*, a marker of bone mineralisation activity, were quantified by qPCR using SYBR green assays (DNA Essential Green Master mix, Roche) and expression levels normalised using the housekeeper gene *RPL37*.
Results: Preliminary results from qPCR analysis indicated a decrease in *BMP7* expression in the vertebral cartilage of pre-weaning kyphotic pigs compared to age matched control pigs. Interestingly, *BMP7* expression reduced from pre-weaning to weaning in healthy pigs (P<0.05) but remained constant for kyphotic pigs in both age groups. The array suggests kyphotic pre-weaners are not achieving sufficient cartilage mineralisation which would otherwise result in normal bone growth and development.
Conclusion: Our initial results indicate reduced bone mineralisation in vertebral cartilage of pre-weaning kyphotic pigs. *BMP7* expression was highest in healthy pigs at pre-weaning, most likely to facilitate bone growth in a rapidly growing period of development. Decreased cartilage mineralisation in kyphotic pre-weaners possibly facilitates widening of intervertebral disc space which conforms the spine into the characteristic hump-back. The results suggest that the highest risk of developing kyphosis occurs during the pre-weaning stage.
This work was funded by the PROHEALTH project and Newcastle University.

Disclosure of Interest: None Declared

Keywords: Cartilage , Kyphosis, qPCR

Poster Abstracts

Welfare and Nutrition

PO-PT2-151

Ultraviolet treated liquid plasma PCR+ for PEDV spray dried and fed to naïve pigs was not infective and performed equal to pigs fed untreated plasma

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Introduction: Spray dried plasma (SDP) is a specialty protein source used in pig diets due of its beneficial effects on post-weaning performance and survival. Processing of SDP produces a safe product; however, further evaluation of redundant safety steps may be investigated. The objectives were to determine the effect of commercially produced ultraviolet light (UV) treated liquid porcine plasma containing PEDV genome with a special UV system developed for turbid liquids and then spray dried (UV-SDPP) and used in a diet for PEDV naïve weaned pigs on performance and PEDV transmission compared to pigs fed a diet without SDP or with spray dried bovine plasma (SDBP).
Materials and Methods: Eighty pigs (21 d of age) were randomized to three treatments (trt) and balanced by BW and gender to provide 4-6 replications per trt (4 pigs/pen). Dietary trt were: 1) control: soy protein concentrate; 2) UV-SDPP: 5% UV treated (2836 J/L) commercial SDPP positive for PEDV genome (qrtPCR Ct = 32); and 3) SDBP: 5% commercial SDBP. All pigs were housed in the same room and air space. All diets contained 20% SBM and 20.6% dried whey, were non-pelleted, non-medicated and formulated to contain 3.4 Mcal ME/kg and 1.45% SID lysine, and fed for 14 d post-weaning. Individual pig BW was recorded at 0, 7, and 14 d post-weaning and average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (F:G) was calculated for these periods. Pigs were evaluated daily for symptoms of diarrhea, respiratory, and body condition. Rectal fecal swabs for each pig were collected at d 0, 3, 7, and 14 and submitted for PEDV qrt-PCR. Pigs were necropsied on d 14 and gross evaluation of tissues noted. Intestinal contents were collected at termination and submitted for PEDV qrt-PCR and tissue samples were subjected to immunohistochemistry (IHC). Terminal blood samples were submitted for PEDV antibodies. Analysis of variance included effect of diet and the covariance of initial BW.
Results: Pigs fed UV-SDPP or SDBP had greater (P<0.05) ADG and ADFI compared to the control diet. No significant differences (P>0.05) were noted for F:G, diarrhea, or respiratory symptom days, or physical condition. Analysis of fecal swabs for D 0, 3, 7, and 14 were all negative for PEDV by qrt-PCR for all trt. IHC of intestine samples and PEDV serum antibodies on d 14 were also all negative for all trt.
Conclusion: Feeding pigs diets containing UV-SDPP positive for PEDV genome did not transmit PEDV to naïve pigs through 14 d post-weaning based on fecal qrt-PCR, IHC, and lack of seroconversion. Performance was similar for pigs fed diet with UV-SDPP compared to SDBP diet and better than control diet.

Disclosure of Interest: J. Campbell Conflict with: Employee, J. Crenshaw Conflict with: Employee, J. Polo Conflict with: Employee, R. Saltzman: None Declared, L. Kesl: None Declared

Keywords: pigs, spray dried plasma, ultraviolet light

Welfare and Nutrition

PO-PT2-152

Field study on social behavior, boar taint and performance of male pigs fattened separately or in mixed groups

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Introduction: Fattening of entire male pigs as a consequence of changed legislation results in new challenges for the pork production. With regard to husbandry the question persists whether separate or mixed housing of male and female fatteners is the most appropriate. In this investigation the effects of different housing regimes on social behavior, performance and boar taint were determined.
Materials and Methods: For the field study 1440 male and female genetically defined fatteners were housed in unisex male (U) or mixed male/female (M) groups. Average daily weight gain (ADG) was calculated for each animal based on individual weight at start and at slaughter. Carcass characteristics were monitored by AutoFOM. Neck fat samples were analyzed for androstenone (A: ELISA) and skatole (S: HPLC). Sensory evaluation of boar taint was done on 180 randomized samples (evenly distributed) by a panel of trained assessors. Social behaviour (sexual and aggressive activities) was monitored during the last 3-7 weeks of fattening by the use of “indicator animals” (2/box; 2 times/week, 10 min). Mixed housed females were tested for pregnancy by ultrasonography directly before slaughter (123.3 kg). Statistical analysis was performed by procedure mixed (SAS 9.1). Differences between LS-means were tested by Scheffe-test p<0.05.
Results: Independent of housing only few aggressive activities were counted and no penis biting was observed during monitoring the behavior. Pigs in mixed groups tended to be more interested in their companions than unisex males. Boars in mixed groups were distinctively snuffling more often (p<0.0267) than separately housed; in general boars tended to bite and fight more frequently than females. When females came in heat at the end of the fattening period their biting frequency increased (r_p=0.37). Regarding sexual activities females tended to be less active. In one of the 374 tested females from mixed groups a pregnancy was detected. In the field trial daily weight gain (U:875; M:867g/d), carcass yield (U:76.7, M:76.5%), ham weight (U:18.0; M:18.1kg), loin weight (U:7.02; M:7.03kg) of males from unisex or mixed groups did not differ. Contents of A and S in neck fat of males were independent of housing. Results of sensory analyses were in good agreement with the chemical analyses.
Conclusion: In this field study separate male and mixed housing of entire male and female pigs are feasible in the fattening period. Due to high daily weight gains the pigs were slaughtered at a comparably young age, i. e. without a longer period of sexual activity.Severe problems in behavior and pregnancies did not occur. Regarding performance and boar taint parameters both management-/housing systems can be recommended.

Disclosure of Interest: None Declared

Keywords: behaviour, boar taint, performance

Welfare and Nutrition

PO-PT2-153

Comparing effects of feed additives on growth, gut microbiota and cytokine and cytokine regulatory gene expression in weaning piglets

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Introduction: Post-weaning complications in piglets are characterized by heightened susceptibility to infection, diarrhoea, atrophy of small intestine structure and reduction in body weight. The ban on prophylactic antibiotics and environmental concerns with usage of excessive zinc demands for identification of safer alternatives to treat post-weaning complications. Feed additives that maintain homeostasis between the intestinal epithelial layer, intestinal microbes and the local immune cells are of high interest as they have implications in both animal industry and human food industry. Some previous evaluation of feed additives like seaweed extracts, inulin and β-glucan in animal trials have provided us a glimpse of the vast bioactivity potential they hold. Therefore, the objective of this experiment was to compare the effects of supplementing weaning piglet diet with zinc oxide (ZnO), milk hydrolysate (MH), yeast β-glucan (BG) and a combination of MH and BG on growth, gut microbiota and cytokine and cytokine regulatory protein gene expression.
Materials and Methods: Forty 21 d old weaned piglets (7.3 ± 0.2 kg) were assigned to either: 1) control diet (CD), 2) CD +3.1 g/kg ZnO, 3) CD + 0.25 g/kg MH, 4) CD + 0.25 g/kg BG or 5) CD + 0.25 g/kg MH + 0.25 g/kg BG for 12 days (n=8). Fecal scores per pen were recorded daily and animals were weighed on days 0, 6 and 12. Following sacrifice on day 12, digesta and colonic tissue samples were collected. Tissue samples were used for qPCR analysis while digesta samples were used to enumerate a selected panel of bacterial colonies using 16s rRNA qPCR technology.
Results: The ZnO group and MH+BG treatment group were associated with a similar improvement of growth compared to CD group ($P<0.05$). While the BG group was associated with an increase in AEEC strains in gut, MH+BG group was associated with a decrease in abundance of AEEC strains ($P<0.05$). Thus, while MH or BG individually were not associated with any major improvement in weaning piglets, a combination of MH+BG improved performance of the piglets similar to ZnO group.
Conclusion: Hence, in this study, addition of milk hydrolysate and yeast β-glucan in feed did not have any major effect on the analysed parameters. However, a combination of milk hydrolysate and yeast β-glucan had an overall positive effect on growth and fecal scores of piglets, similar to what was observed in animals supplemented with zinc oxide.

Disclosure of Interest: None Declared

Keywords: feed additive, milk hydrolysate, post weaning diarrhoea

Welfare and Nutrition

PO-PT2-156

Provision of straw by a foraging tower – effect on tail biting in weaners and fattening pigs

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Introduction: Straw is one of the most effective routing materials to reduce tail biting in pigs. A so called foraging-tower (FT) enables to provide only small quantities of straw compatible with liquid manure systems. The focus was on the effect of providing straw by FT for the prevention of tail biting in tail docked pigs.
Materials and Methods: The study was conducted from June 2013 to August 2014 in a conventional farrow to finish herd in Germany, which was affected by tail biting in fattening pigs for several years. Four consecutive batches of 160 pigs each were followed up from weaning to slaughter. The piglets were vaccinated against PCV2 and *Mycoplasma hyopneumoniae* (MH). At weaning the pigs were randomly selected for the treatment (TG) or control (CG) group. During the rearing period the pigs were housed in the same unit (8 pens/20 pigs per pen) and later in one unit of the fattening barn (16 pens/10 pigs per pen). The pens for TG were equipped with a FT permanently providing an adjusted amount of short-chopped straw. In the pens for the CG an equally sized dummy without straw was installed. Once a week the tail of each pig was scored using the parameters “tail damage” and “blood freshness”. The ammonia content in the air was measured at different locations in the unit and the air temperature was recorded permanently. At the beginning and at the end of the fattening period blood samples were taken from 22 randomly selected pigs and tested by PCR for PRRSV and PCV2 as well as by ELISA for antibodies against PRRSV, SIV and MH. The feed and straw consumption as well as the weight gain of the pigs per pen were recorded.
Results: Tail biting was observed in two pens of the CG (14 pigs) and one pen of the TG (4 pigs) of the second batch during the fattening period. The outbreak was probably caused by a sudden increase of temperature (difference of 11.2°C within one day) and ammonia (30-40ppm) due to a failure of the ventilation system. Across all batches up to 8% of the pigs in each group showed bite marks, but this did not result in tail biting. Serology and PCR reveal infections with SIV and MH in all batches and a circulation of PRRSV (NA-vaccine strain) and PCV2 in two batches each. The average daily straw consumption was 3.7g/pig during the rearing period and 31.9 g/pig during the fattening period. Comparing the daily weight gain and the feed consumption of the TG and CG no considerable differences were detected.
Conclusion: Due to the low prevalence of tail biting in all batches the effect of the FT tower could not conclusively be evaluated. The operation of the FT with an adjusted supply of straw did not affect the weight gain and feed consumption.

Disclosure of Interest: None Declared

Keywords: ammonia, SIV, straw

Poster Abstracts

Welfare and Nutrition

PO-PT2-157

Evaluation of urinary bone turnover metabolites as indicators of phosphorus status in sows

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Introduction: Phosphorus (P) is an important component in several processes and the main component of the inorganic bone matrix together with calcium (Ca).The minerals can be released from the bones to the blood by the bone turnover processes associated also breaking down the organic matrix of the bone. For reproducing sows, the physiological demand for nutrients covers the supply to the growing fetuses and eventually for milk production. If the dietary supply of P and Ca does not support the physiological need, the P and Ca pool in the bones will be released. The release of minerals from the bone tissue is a normal procedure and a sign of a healthy bone function, however if the dietary supply does not meet the demand of the sow, the mineral release form the bone tissue (bone turnover) will be intensified above normal resulting in a high drainage of the bones making them more fragile. Breakdown metabolites of bone turnover can be measured in the urine and these constituents may be a useful tool to evaluate the physiological P status of the sow.
Materials and Methods: 60 pigs were included in the experiment and randomly distributed to three groups. The animals were entering the experiment as gilts of 50 kg BW and the experiment continued throughout two reproductive cycles. The experimental diets differed in the dietary content of total P: Low (mean 3.5 g/kg), Medium (mean 4.1 g/kg) and High (mean 4.7 g/kg). All other nutrients and energy were according to Danish recommendations and were identical for all treatment. Urine samples were collected in early and late pregnancy, at farrowing and every week during lactation (4 week). The urine samples were analyzed for the metabolites pyridinoline (PYD), deoxypyridinoline (DPD) and carboxyterminal cross-linked telopeptide of type I collagen (CTX) by LC-MS/MS.
Results: In general, the preliminary results show that the excretion of bone markers depends on the sows' physiological stage as the urinary concentration decreased from late pregnancy until farrowing followed by and increase during lactation. Irrespective of dietary P supply, the excretion of metabolites seems to reach a peak in early pregnancy in the reproduction cycles. The results indicate that the urine concentration of the three markers were highest when the sows were fed reduced dietary P.
Conclusion: The concentration of bone metabolites quantified in urine seems to be a promising measure to evaluate the physiological P status of sows.

Disclosure of Interest: None Declared

Keywords: bone metabolites, phosphorus, sow

Welfare and Nutrition

PO-PT2-164

EFFECT OF THE NUTRITIONAL SUPPLEMENT VIUSID vet, ON THE PRODUCTIVE BEHAVIOR OF LACTATING SOWS AND THEIR PIGLETS.

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Introduction: In a swine breeding, the piglets are those which require more nutritional care due to their physiologic immaturity and the enzymatic changes that take place in these first stages of life. The objective of this experiment was to assess the effect of the nutritional supplement VIUSID vet on the productive behavior of lactating sows and their piglets.
Materials and Methods: For this work 54 Yorkshire lactating sows and 548 piglets were used. They were distributed in four homogeneous groups. Group I, was used as control. Group II was supplemented with 10 grams of Viusid vet per animal, from 104 days of pregnancy until the weaning (26 days). Group III: in this group each piglet was given 2 g of Viusid vet per kg of concentrate food (from 10 days of age and until the weaning). The Group IV consisted in a combination of the treatments II and III.
The main variables studied were: Size of the litter at weaning; weight of the piglet at weaning; increment of weight, daily mean gain, alimentary efficiency, weigh final of the litter, mortality in piglets. Also percent of piglets that classify to the following category, born alive and dead in the following farrow and Weigh to the birth of the next farrow.
VIUSID vet contains: Malic acid, Glucosamine, Arginine, Glycine, Ascorbic Acid, Folic Acid, Monoammonium Glycyrrhizinate (extracted of the root of *Glycyrrhiza glabra*), Pyridoxine Hydrochloride, Cyanocobalamin, Calcium Pantothenate and Zinc Sulfate. The product undergoes a biocatalytic process of molecular activation to improve their biological activity and the biochemical reactivity of all their molecules.
Results: It was observed that the VIUSID improved significantly ($P<0.05$), the indicators of gain weight and of the alimentary efficiency of the piglets, in the supplemented groups as compared to the control. Group IV had the best behavior, with a gain of weight higher than 15.87% and an improvement of the alimentary efficiency of 19.40%, as compared to the control group. It was found an increment of 22% in the weight of the litter, with respect to the control group. This enhancing effect may be increased when the supplement is provided both to the mother and to the breeding. The groups II and III did not differ from each other. The selection of piglets as future breeders was 20-24 % higher in the supplemented groups. The other variables did not differ statistically.
Conclusion: The dietary supplement VIUSID vet, improves the productive behavior of the lactating sows and their piglets. The best effect is obtained when both receive the supplement.

Disclosure of Interest: None Declared

Keywords: Molecular activation, piglets, viusid

Welfare and Nutrition

PO-PT2-165

EFFECT OF THE DIETARY SUPPLEMENT VIUSID vet, ON THE PRODUCTIVE BEHAVIOR OF NEWLY WEANED PIGS.

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Introduction: The weaning is an important period in the life of the pigs, because they require adapting quickly to the environmental and nutritional changes that exist in the pigpen. The objective of this assay was to assess the effect of three dose of the dietary supplement VIUSID vet, on the productive behavior of pigs after the weaning.
Materials and Methods: For this work four groups of 30 newly weaned pigs were used (females). The animals were weaned at around of 26 days of age. The assay lasted 60 days.
The pigs of the groups I, II and III were supplemented with VIUSID vet, in dose of 1, 1.5 and 2 grams per kg of concentrated food, respectively. The group IV was used as control.
The main variables studied were: Initial weigh, Final weight, Increment of weight, Average daily gain, Alimentary efficiency, Incidence of diarrheas (%) and Viability (%).
The variables were processed statistically according to the test T for unequal variances, except the incidence of diarrheas and the viability, which were processed by the proportion hypothesis test.
VIUSID vet contains: Malic acid, Glucosamine, Arginine, Glycine, Ascorbic Acid, Folic Acid, Monoammonium Glycyrrhizinate (extracted from the root of *Glycyrrhiza glabra*), Pyridoxine Hydrochloride, Cyanocobalamin, Calcium Pantothenate and Zinc Sulfate. The product undergoes a biocatalytic process of molecular activation to improve their biological activity and the biochemical reactivity of all its molecules.
Results: The increment of weight and the alimentary efficiency, were higher ($p < 0.05$), in the pigs supplemented with VIUSID vet. The groups tried with 1 and 1.5 grams had increments of 6 and 11% for the average daily gain, and of 6 and 12% for the alimentary efficiency, compared with the control group. The treatment with 2 grams of VIUSID vet, improved the gain weight in 5.3%. The incidence of diarrhea was of 2.5, 7.5 and 2.5% in the pigs of the groups I, II and III, respectively, vs. 15% in the control group. The viability did not differ among the groups.
Conclusion: The supply of 1.0, 1.5 and 2.0 grams of VIUSID vet. per kg of concentrated food, significantly improves the gain weight and the alimentary efficiency of the weaned pigs; besides, reduces the incidence of diarrheas.

Disclosure of Interest: None Declared

Keywords: Molecular activation, viusid, weaned pigs

Welfare and Nutrition

PO-PT2-166

Farrowing liveweight and ADGW in litters from sows treated with Metacam

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Introduction: Farrowing is a critical period for the sow in which painful, inflammatory and infectious processes along with other systemic events cause post-partum stress and may trigger Postpartum Dysgalactia Syndrome (PPDS) which manifests itself either in clinical or subclinical presentation, both with negative impact on the sow and its litter regarding the growth development of suckling pigs. NSAIDs are a pharmacological group widely used in veterinary medicine due to their anti-inflammatory, analgesic and antipyretic properties. Among these Metacam with its active ingredient meloxicam - a COX-2 selective inhibitor - applied at the end of farrowing has proven to be effective and safe on PPDS control, improvement of sow welfare and behavior, and overall milk and colostrum intake for piglets during the first days of life. The objective of this study was to determine under field conditions the effect of meloxicam in litters from sows of a farm with subclinical PPDS in terms of productive performance of their litters liveweight and ADGW at weaning.
Materials and Methods: The study took place on a multi-site intensive farm and it considered a total number of 265 sows and 3077 piglets. Sows were divided into two experimental groups, Group A (n=127 sows) was treated with Metacam 5 ml IM and Group B (n=138 sows) was injected with saline solution 5 ml IM to serve as non-treated control group. One hour after farrowing each piglet was individually identified with numbered ear tags and cross fostering was restricted to first 24 hours within litters from a same treatment group. A research coordinator monitored the experience from farrowing to weaning. The experimental was the litter. A lineal general univariate model was used to assess statistical differences between groups for liveweight at weaning and ADGW during the farrowing period.
Results: Piglets in the meloxciam group presented a greater live weight at weaning with 0,28 kg of advantage compared to control group. LS Means for live weight at weaning where 5,23 for meloxicam group (n 1504) and 4,95 for control group (n 1573).
ADGW across the whole farrowing period was superior in the meloxicam group with 13 g daily advantage over the control group. LS Means for site 1 ADGW where 0,242 for the meloxicam group (n 1504) and 0,229 for the control group (n 1573). This difference was statistically significant ($p < 0,05$).
Conclusion: The litters from sows treated with Metacam had a better growth performance from birth to weaning (21 days average). These results are in line with previous research and further confirm the value of controlling lactation disorders during the first critical hours for piglets colostrum and milk intake.

Disclosure of Interest: C. Gutiérrez Conflict with: Boehringer Ingelheim, D. Guiñez: None Declared, V. Grosse-Liesner: None Declared, A. Ruiz: None Declared, C. Roudergue Conflict with: Boehringer Ingelheim

Keywords: Meloxicam, Metacam, NSAID

Poster Abstracts

Welfare and Nutrition

PO-PT2-167

Efficacy of straw-filled rooting tower for prevention of tail injuries in fattening pigs

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Introduction: Tail biting outbreaks rate among the major threats in pig production. To prevent or at least minimize tail biting docking is routine practice in Germany, although it contradicts the Council Directive 2008/120/EC. Many factors, like climate, general health status, stocking density, food and water supply and especially insufficient environmental enrichment have a major impact on the tail biting incidence. In the present study, a straw-filled rooting tower ("Düsser Wühlturm") was tested for avoiding tail biting in undocked fattening pig groups.
Materials and Methods: The study was performed in a conventional fattening farm during three fattening periods. A total of 325 pigs with undocked tails ware observed until slaughter using a tail scoring system and a partial behavioral ethogram. In 8 pens straw-filled and movable rooting towers and in 5 pens empty and fixed rooting towers ("dummies") were installed to provide the same place for every pig. 25 pigs were allocated in a pen. During the first ten weeks of fattening period, tails were scored once a week, while behavior of pig groups was analyzed every second week by video surveillance covering 110 minutes per day. Evaluated parameters were: interactions a) with the rooting tower, b) with stable enrichments, c) with other pigs head, d) with other pigs torso, e) with other pigs tail, and f) with other pig legs.
Results: Tail biting outbreaks occurred in 25 % of pens equipped with a straw-filled rooting tower and in 80 % of pens with a dummy. No differences in the kind of tail lesions (deep skin lesions, tail losses, acute bleeding, swelling) were observed. Neither significant differences between both groups were found summarizing interactions on all body regions. Compared to tail manipulation, head manipulations were recorded most often especially during feeding as well as in periods between feeding times in pens with the straw-filled rooting tower ($p=0.03$). In general, more interaction with the straw-filled rooting tower was observed than with the dummy.
Conclusion: The straw filled rooting tower ("Düsser Wühlturm") combines advantages of a spatial structuring object and enrichment. Although it is of interest for pigs during the whole fattening period, in this study it was found to be not suitable for prevention of tail biting in fattening pigs with undocked tails.

Disclosure of Interest: None Declared

Keywords: environmental enrichment, tail biting, tail docking

Welfare and Nutrition

PO-PT2-170

EFFECT OF THE NUTRITIONAL SUPPLEMENT VIUSID vet, ON THE PRODUCTIVE BEHAVIOR OF FATTENING PIGS.

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Introduction: The correct feeding of pigs in the fattening phase is a difficult challenge for any nutritionist. Due to the rapid advances in the use of new and diverse preservatives and ingredients, the existence of new ways of exploitation and technologies, as well as for the demands of a market increasingly informed and interested in consuming products of animal origin of good quality, at an accessible price. The objective of this assay was to assess the effect of the nutritional supplement VIUSID vet on the productive behavior of fattening pigs.
Materials and Methods: For this work, 58 pigs in fattening phase were used. Three homogeneous groups were delimited according to corporal mass, sex and physical condition. Each pig was considered an experimental unit. The treatments consisted of: Group I (19 pigs): Control group. Group II (20 pigs): This group received VIUSID vet, in a dose of 1.5 g/kg of concentrated food daily. Group III (19 pigs): This group received VIUSID vet, in a dose of 1 g/kg of concentrated food daily. The assay lasted 122 days. A simple ANOVA test, after the confirmation of a normal distribution (Kolmogorov-Smirnov test) and an equality of variances (Levene test) were applied. As there were significant differences, the Duncan´s multiple range test was used.
The nutritional supplement Viusid vet, contains: Malic acid, Glucosamine, Arginine, Glycine, Ascorbic Acid, Folic Acid, Monoammonium Glycyrrhizinate (extracted from the root *Glycyrrhiza glabra*), Pyridoxine Hydrochloride, Cyanocobalamin, Calcium Pantothenate and Zinc Sulfate. The product undergoes a biocatalytic process of molecular activation to improve their biological activity and the biochemical reactivity of all its molecules.
Results: In the experiment it was observed that the animals supplemented with VIUSID vet, in a dose of 1.5 g/kg of concentrated food daily, improved significantly ($P<0.05$) the final weight (4.5 kg more), the daily mean gain weight (12.16% more) and the alimentary conversion (3.08 vs 3.76), as compared to the control group. Group II, supplemented with VIUSID vet, in dose of 1 g/kg of concentrated food, did not differ from the control group. There were neither deaths nor diarrheas during the experiment.
Conclusion: The dietary supplement VIUSID vet, in a dose of 1.5 g/kg of concentrated food significantly increased the weight gain and the alimentary conversion of the fattening pigs studied.

Disclosure of Interest: None Declared

Keywords: Molecular-activation, pigs, Viusid

Welfare and Nutrition

PO-PT2-178

POSITIVE INFLUENCES ON STOMACH HEALTH DUE TO FEEDING A 2-STAGE COARSELY GROUND DIET IN YOUNG PIGS

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Introduction: More than 80% of slaughter pigs in England show epithelial changes at the *Pars nonglandularis* (PN) of the gastric mucosa. In the interest of animal welfare and to avoid prospective cost deductions, alternative pig feeding strategies must be developed. In this study the effects of a 2-stage coarsely ground compound feed (COF) on performance and stomach health of piglets were tested as compared to a conventional 1-stage produced COF.
Materials and Methods: Over a period of 4 weeks 10 weaned barrows (age: 40 days; Body weight: 8.90 ± 1.00 kg) were housed individually and offered a botanically and chemically identical diet
(15.6 MJ ME, 210 g CP per kg DM), which differed in grinding type and intensity. COF_H was produced conventionally, finely ground by hammer mill and fed to controls (n=5). Ingredients of COF_{MH} for the second group of piglets (n=5) were ground using a multicracker (first step). After sieving, only coarse particles (> 2500 microns) were ground by hammer mill (second step). Both diets were offered as dry mash. The mucosa of the PN was evaluated by a macroscopic scoring system at the trials´ end at necropsy.
Results: COF_H had a geometric mean diameter (GMD) of 395 microns and COF_{MH} had a GMD of 576 microns. Regarding performance parameters (daily feed intake, daily gain, feed conversion ratio) there were no differences (P>0.05) between both groups, although COF_{MH} showed the most favourable results. Furthermore, the region of PN piglets of group COF_{MH} did not show any signs of mucosal alterations, while in piglets of group COF_H low-grade hyperkeratosis was detected.
Conclusion: In comparison to conventional hammer mill diminution, 2-stage grinding by multicracker followed by hammer mill reduces energy costs by about 30% and showed in this study no negative effects on performance but distinct advantages on gastric health. There are epidemiological studies in Europe but also from worldwide indicating that the majority of slaughtered pigs are affected by gastric ulcers/alterations of the PN. Not only for prevention of gastric mucosal lesions, but also from an economic and ecological point of view producing and feeding of 2-stage coarsely ground COF seem to be suitable.

Disclosure of Interest: None Declared

Keywords: None

Welfare and Nutrition

PO-PT2-179

Field study on effects of grinding intensity of feed on performance, androstenone and skatole content in neck fat of male and female finishing pigs

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Introduction: The castration of male piglets without anesthesia is under public pressure and is already forbidden in some countries. The fattening of boars is an alternative but due to the accumulation of androstenone (A) and skatole (S) in the fatty tissue, resulting in the so called boar taint, some carcasses may be condemned and therefore excluded from human consumption. Skatole is a product of the microbial tryptophan degradation in the hindgut, which can be lowered by feeding raw potato starch or inulin. Aim of this field study was to find a feeding concept to minimize skatole formation in the digestive tract of pigs. Therefore coarse grinding of a cereal based diet was chosen to reach a higher starch influx into the hindgut mimicking the effects of raw potato starch.
Materials and Methods: The study was performed with a total of 490 male (m) and 178 female (f) finishers receiving either a finely (F; n=325) or a coarsely ground diet (C; n=343) based on the same ingredients for the last 3-7 weeks of fattening. Diet`s structure was characterized by wet sieve analysis resulting in a different geometric mean diameter (GMD) of 598 µm (F) and 700 µm (C). Twenty pooled fecal samples were analyzed for dry matter (DM), starch content and pH value. Average daily weight gain (ADG) was measured individually over the whole fattening period (27.8-124.9 kg) and feed conversion ratio (FCR) was calculated. Carcass characteristics were monitored by Auto FOM, neck fat samples analyzed for A (ELISA) and S levels (HPLC). Statistical analysis was performed by procedure mixed (SAS 9.1). Differences between LS-means were tested by Scheffe-test p<0.05.
Results: DM and pH values were significantly reduced in feces of C fed pigs (DM g/kg: C 243, F 264; pH: F 7.24, C 6.99), whereas the starch content was significantly higher (g/kg DM: C 54.6, F 43.3). Feeding regime had no influence on androstenone (ng/g; male: F 1256, C 1236; female: F 171, C 161) or skatole levels (ng/g; male: F 92, C 105; female: F 35, C 38) in the neck fat. Neither ADG of males (g/d: F 890; C 891) and females (g/d: F 854; C 850) nor FCR (both sexes: C 2.47 vs F 2.51) differed between the groups. Under both feeding regimes carcass yield (male: C 75.8%, F 76.7%; female: C 78.6%, F 79.3%), ham weight (kg; male: F 18.1, C 18.0; female: C 18.5, F 18.4) and loin weight (kg; male: F 7.07, C 6.99; female: C 7.26, F 7.23) were lower for the boars.
Conclusion: Although the diet was able to change important fecal parameters no effects on skatole and androstenone contents in neck fat were achieved. Regarding fattening performance, advantages of higher daily weight gain of the boars were not reflected in carcass yield due to the weight of testicles.

Disclosure of Interest: None Declared

Keywords: boar taint, grinding intensity, performance

Poster Abstracts

Welfare and Nutrition

PO-PT2-187

Various levels of milk by-products in weaning pig diet on growth performance, blood profiles, carcass characteristics and economic analysis

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Introduction: It is well known that lactose and whey powder were used as the main raw materials in weaning pig diet due to the fact that supplementation of milk by-products in weaning pig diet helped maintaining an enhanced intestinal environment in pig. Moreover, increasing of growth and enhancing feed intake of weaning pigs by supplementation of milk by-products has been known widely in weaning pigs' diet. However, high levels of dietary milk by-products induced the increment of feed cost, resulted in a great burden for swine producers. Therefore, this experiment was conducted to evaluate various levels of milk by-products in weaning pig diet on growth performance, blood profiles thereafter later growth, carcass characteristics and economic analysis of finishing pigs to figure out carry-over effect.
Materials and Methods: A total of 160 weaning pigs ([Yorkshire × Landrace] × Duroc), average 7.01 ± 1.32 kg BW, were allotted to one of four treatments by BW and sex in 10 replications with 4 pigs per pen in a randomized complete block (RCB) design. Pigs were fed each treatment diet with various levels of milk by-products (Phase 1: 0, 10, 20 and 30%, Phase 2 : 0, 5 10 and 15%), respectively. Six phase feeding programs were used in the whole experimental period. Blood profiles and meat quality were evaluated. Economic analysis was calculated using amount of the total feed intake and feed price. Data was analyzed by using the GLM procedure of SAS.
Results: In feeding trial, BW, ADG and ADFI of weaning pigs were declined as low levels of milk by-products diets were provided, resulting in linear response ($P<0.05$). However, improved growth response by high dietary milk by-products were disappeared in growing and finishing phases and significant difference was not observed among treatments at the end of experiment. The BUN concentration, IgA and IgG had no significant differences among dietary treatments. Pork color, pH and proximate analysis of longissimus muscle did not show differences among treatments. When pigs were fed no milk by-products treatment diet during weaning period, feed cost to 110 kg of market weight was decreased approximately 11% compared to high milk by-products treatment.
Conclusion: Various levels of dietary milk by-products in weaning pig's diet influenced on BW, ADFI and ADG in weaning pig period but subsequent growth performance of growing-finishing pig was not affected by dietary treatments. When lower level of milk by-products was supplemented in weaning pig's diet, feed cost was decreased without any negative effect in growth performance during weaning to finishing period.

Disclosure of Interest: None Declared

Keywords: milk by-products, weaning to finishing pigs

Welfare and Nutrition

PO-PT2-195

A survey of tail docking at pig farm in Korea

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Introduction: In swine industry, piglets are tail docked at 3~7days old to prevent tail biting which is not fully understood what reason is. However, in the point of animal welfare, the cutting action is on negative perspective. Because the tail is a key role in communicating each pig and expressing their conditions, and docking practice causes amputation neuroma which is accompanied with pain. The aim of this survey was to examine the length and the diameter of docked tails for indicator to animal welfare at pig farm in Korea.
Materials and Methods: At slaughterhouse, a total 600 tails were surveyed about length and diameter according to methods by M.S. Herskin *et al.*, 2014. In their research, the intact tail length of crossbred LYD (Landrace x Yorkshire x Duroc) was 30.6 ±0.6 cm (n=65). The length of docked tail was a distance betwefen 1cm from the root and 0.5cm from the tip, and the diameter was at the 0.5cm from the tip. The experimental design was divided into 3 groups: group L (long, 15.3 cm ≤ length < 22.95 cm, 25~50% docking), group M (moderate, 7.65 ≤ length < 15.3, 50~75% docking) and group S (short, length < 7.65, 75~100% docking). Tail lengths and diameters were measured by a ruler, and docking percentages were estimated through the referred intact tail length, mentioned above. Kruskal-Wallis test of SPSS statistics 21 (IBM Corp., USA) were used for relationship between lengths and diameters in each group.
Results: Total tail mean length was 11.31±4.00 cm, mean diameter was 1.30 ± 0.16 cm and docking percent was 63.01 ± 12.06%, respectively. The results of tail lengths, diameters and docking percentage in each group as follow: group L (n=120, 20%) was 17.80 ± 1.40; 1.11 ± 0.11 and 41.78 ± 4.57%, group M (n=367, 61.17%) was 10.69 ± 1.91; 1.31 ± 0 and 65.03 ± 6.25%. and group S (n= 113, 18.83%) was 6.43 ± 0.92; 1.46± 0.07 and 78.88 ± 3.03, respectively. In relationship between lengths and diameters of each group, the diameter of L group was respectively smaller than the other groups ($P <$ 0.001).
Conclusion: These results show that the docking practice in Korean pig farm tended to remove 50~75% of the tails. The observed differences in tail length and diameter at the time of slaughter may be useful as welfare surveillance tools in order to identify the docking practice in pig farms.

Disclosure of Interest: None Declared

Keywords: animal welfare, Pig, tail docking

Welfare and Nutrition

PO-PT2-196

A study of average feeding time, in relation to parity, in large group gestations with EFS.

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Introduction: Animal welfare, has been traditionally evaluated by measuring level of aggression and stress parameters. However, feeding behavior can also provide a valuable indication of comfort or stress for gestating sows in large groups, when fed with ESF systems.
Materials and Methods: The study was conducted at Albesa-Ramadera a 3300 sow, Site 1 farm, based in Catalonia, Spain. The farm has large group gestation (128 to 175 sows per group) and utilizes Electronic Sow Feeding Stations (ESF). Nulliparous sows are placed separated in dynamic pens, while all other multiparous sows, (parities 2-7) are placed in larger dynamic groups. All pens have two ESF, per pen.
During the one week period of this study, the farm had, what could be considered average production parameters considering its present health status and genotype.
ESF were pre-programmed to open (start feeding) at 00:10 AM every morning and closed at 23:50 PM.
Two multiparous pens (pen 2, and 6) were considered in the study.
The productive entry time (entry in which the sow is fed) of every sow in the pen was recorded. This data was then correlated to the sow's parity.
The data was analyzed by a linear model (GLM, SAS, JMP pro.), considering the dependable variable the AET, and as the systemic variable, the parity of the sow. To compare the AET, within parity's the t student test was used, and significance levels were established at; $p < 0.05$.

Results:
In multiparty pens, sow were grouped considering their average time of entry to the ESF. Average entry time and variation coefficient were considered for every parity group, as seen on the Graff 1. Table 1, shows; parity group size, AET, and the significance of the difference. Different letters mean differences were significant.
It can be seen from the data recorded from both pens, that sow of higher parities, enter to feed first in the day, as lower parities tend to wait until these sow have fed.
Conclusion: These results, show how sow sub-group within large pens, and feed following a dominance order. Sows from older parities (5-7) if in small numbers, tend to enter more or less together. The parameters measured could be considered useful to evaluate normal or abnormal feeding behaviors especially within farm comparisons. However they should not be considered universal, since, large differences are observed when considering other types of ESF, different degree of sow training, group size, parity structure, time in gestation or amount of feed administered.

Disclosure of Interest: None Declared

Keywords: Electronic sow feeding behaviour

Welfare and Nutrition

PO-PT2-197

Supplementation with Clostridium butyricum (Miya-Gold® S) improves feed conversion and daily gain

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Introduction: The first Danish field experience with *Clostridium butyricum* (Miya-Gold® S) supplementation in feed is described in an integrated sow and 7-30 kg weaner pig farm with home mixed feed. Feed conversion and average daily gain were monitored 3 months before (4328 pig sold) and 4 months after (6421 pigs sold) the start of probiotic supplementation.
Materials and Methods: The case farm is SPF producing 22.000 7-30 kg pigs from own sow herd and vaccinating for *Porcine Circovirus Virus* type 2. Pigs were affected by respiratory infections caused by Pandemic swine influenza during the winter and spring 2015. Enteritis was present, however not causing high mortality, but reducing pig performance. As the farm had a history of *Salmonella* confirmed by laboratory reports, it was decided to include Miya-Gold® S in the last week of July 2015 to improve performance. The first feed after weaning was a continuation of the creep feed used in the farrowing crates before switching to a (6-9 kg) homemade meal feed with 3000 ppm zinc oxide. Miya-Gold® S was included at 2 kg /MT in the first (6-9 kg) feed, 1 kg /MT in the second (9-15 kg) feed and 0,5 kg/MT in the third (15-30 kg) feed. The last feed (25-35 kg) did not contain Miya-Gold® S. Inventory of pigs and calculations were based on weekly recordings in the stable, and invoices of numbers and kg pig sold per week. The inventory was adjusted for own production of gilts assigned a weight of 30 kg. Feed production of the different feeds in kg was extracted from records in the milling software. Piglets were assigned a standard weight of 6 kg at weaning. Energy calculations were based on recipes from the mineral premixer. Pigs are held 53 days at the site on average.
Results: Average daily gain improved from 414 g/day to 499 g/day. Feed conversion improved from 1,86 kg feed/ kg gain to 1,81 kg feed/kg gain, measured in energy from 2,06 FEs/kg gain to 1,81 FEs/kg gain. Direct cost saving amounts 0,45 DKK per kg produced equal to 0.06 € per kg.
Conclusion: Control of intestinal health is crucial for piglet performance. Miya-Gold® S supplementation delivers a 10% cost saving on feed in this case with the benefit of a better flow through the post weaning facilities.

Disclosure of Interest: None Declared

Keywords: Clostridium butyricum, pig, zootechnical parameters

Poster Abstracts

Welfare and Nutrition

PO-PT2-208

Effects of different dietary P levels on performance and bone health in weaned piglets

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Introduction: Phosphorus (P) belongs to the essential elements in animals` nutrition and is responsible among others for performance and bone health in weaned piglets. Nowadays, phosphorus achieves an economic relevance due to limited resources of this element. Therefore, aim of the present study was the evaluation of effects of different phosphorus levels in the diet on the performance and bone structure and composition as well to assess bone health in weaned piglets.
Materials and Methods: Diets based on wheat, barley and soybean meal with varying levels of soluble phosphorus (0.30 / 0.47 / 0.66 % in dry matter) were fed to piglets from weaning (28 days p.n.) until slaughter (64 days p.n.). Within this time, body weight gains, feed intake, feed conversion rate, blood parameters and fecal quality were evaluated. The right femur of the slaughtered piglets was scanned with a Skyscan 1076 micro-CT. Trabecular bone mineral density (BMD), cortical tissue mineral density (TMD) and specific microstructural parameters were determined as well. The structure of the bone was examined using a scanning electron microscope (SEM) coupled with an energy dispersive X-ray spectroscopy (EDX).
Results: Feeding a diet with a soluble phosphorus level of 0.66% resulted in a decreased feed intake and lower body weight gains and higher feed conversion rates in consequence. Serum concentration of inorganic phosphate, calcium and magnesium decreased in this group, too. A reduction of the phosphorus level in the diet to 0.30% did not result in an insufficient BMD of the bones. According to preliminary biomechanical results, bony strength seems not to be influenced by any of the tested diets.
Conclusion: A reduction of the phosphorus level in diets for weaned piglets below usual recommendations was not combined with negative effects on the bone structure and should be discussed to spare phosphorus resources.

Disclosure of Interest: None Declared

Keywords: None

Welfare and Nutrition

PO-PT2-209

Effect of sow parity and other lactation parameters on pig welfare lesions in the weaner and finisher stages

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Introduction: Piglets from gilts have poorer performance and health during later life. The objective of this study was to evaluate the effect of sow parity and other lactation parameters on pig welfare lesions in the weaner and finisher stages.

Materials and Methods: The study was conducted on a farrow-to-finish commercial farm. At birth, the sex and bodyweight (BW) of 847 piglets was recorded as well as the parity of their dam and the number of piglets born alive in their litter. The number of times each piglet was transferred between different sows was recorded during lactation as well as the total suckling period for each pig. At weaning and at each subsequent move between the weaner and finisher stages pigs were inspected individually by a single observer for the presence (score = 1) or absence (score = 0) of tail, ear, and body lesions. Data were analysed using binomial logistic regression in PROC GENMOD of SAS v9.3. There were few sows of parity ≥ 6 so parity was reclassified as parity 1 to 5 and parity 6+. Models included parity, sex, no. of transfers and production stage as fixed effects. Lactation length, BW at birth and number of piglets born alive were included as linear covariates.
Results: Parity was a significant source of variation for tail and body lesions ($P < 0.05$). Pigs born from sows of parity 6+ were 1.9 times more likely ($P < 0.05$) to have tail lesions later in life when compared with parity 1 sows. There was no difference in the likelihood of having tail lesions later in life between parity 1 and parity 2 to 5 sows ($P > 0.05$). Pigs born from parity 2, 3 and 4 sows were 2.1, 2.2 and 1.8 times more likely to present ear lesions later in life compared to pigs born from parity 1 sows ($P < 0.05$). No relationship between length of lactation or between the number of piglets born alive and the presence of welfare lesions was observed ($P > 0.05$). The risk of having tail lesions later in life increased by 0.8 times for each 1 kg increase in BW at birth. Body weight at birth was not related with the risk of ear or body lesions later in life ($P > 0.05$). One-hundred-and-forty-seven pigs were transferred from their dam to a different sow and 53 pigs were transferred more than once during lactation. There was no relationship between number of transfers and tail or body lesions ($P > 0.05$) but pigs that were transferred once were 1.7 times more likely to have ear lesions in the subsequent production stages ($P > 0.05$).
Conclusion: Pigs born to parity 1 sows were at no greater risk of welfare lesions during the production stages and indeed were less likely to incur ear lesions. Transferring piglets between sows may place them at higher risk of ear lesions in later life.

Disclosure of Interest: None Declared

Keywords: Lactation parameters, welfare lesions

Welfare and Nutrition

PO-PT2-213

AFLATOXIN STATUS OF SOME SWINE FEEDS IN IBADAN NIGERIA

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Introduction: The use of industry by-products and few other feed ingredients as feedstuff for swine is a regular practice and usually increases animal performances, hence making it more economical. The occurrence of aflatoxin contamination is global, causing severe problems especially in developing countries. Due to the insidious nature of aflatoxin production and the resulting disease states which made diagnosis of aflatoxin difficult; many cases of animal aflatoxicosis have often not been reported in Nigeria. This suggests that little has been done on mycotoxicosis in Nigeria and there is paucity of information on aflatoxin in swine feed as there is no data for the co-occurrence of aflatoxins in swine feed in the country. The aims of the study was to provide information on the aflatoxin status of swine feeds and to identify the aflatoxin types found in swine feeds.
Materials and Methods: Twenty-two samples of the swine feeds were collected from different farms at different occasions. High performance liquid chromatography was used for separation and quantification of aflatoxin fractions in the feed samples.
Results: Results indicate that aflatoxins B1, B2, G1 and G2 were detected in all the samples investigated, with B1 being the most abundant across the samples except for one of the samples in which aflatoxin G1 is the most abundant across the sample.
The range of concentration of total aflatoxins was 42.20 to 184.25 µg/kg (mean: 109.39 µg/kg). The results of this study shows that swine feeds in Ibadan, Nigeria are frequently contaminated with aflatoxins at levels that are considered to be capable of inflicting some level of acute poisoning to the pigs that consume such feeds. Aflatoxin levels in the samples were higher than the recommended limits (20 ppb) for complementary feedstuffs in all animals.
Conclusion: The results of this study shows that swine feeds in Ibadan, Nigeria are frequently contaminated with aflatoxins at levels that are considered to be capable of inflicting some level of acute poisoning to the pigs that consume such feeds. Aflatoxin levels in the samples were higher than the recommended limits (20 ppb) for complementary feedstuffs in all animals.

Disclosure of Interest: None Declared

Keywords: Aflatoxin, Documentation, Swine Feed

Welfare and Nutrition

PO-PT2-215

Oral supplementation of newborn piglets with Lianol® Colostro to reduce early mortality

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Introduction: The aim of the experiment was to study the effect of a complementary feed product orally administered to newborn piglets on their survival and growth: Lianol® Colostro, a pro-metabolic regulator derived form fermented potato protein.
Materials and Methods: The experiment was performed on a commercial swine herd in Thailand. 43 litters (a total of 518 piglets) were distributed according sow's parity to treatments: CONTROL group (n=23 litters), no oral supplementation to piglets; LIANOL group (n=20 litters), small piglets (SP: birth BW ≤ 1.35kg) received 1 ml Lianol® Colostro just after birth and a 2nd one 8h later. Piglets were weighed after birth, at 24h and at day 21 of life. Piglet's rectal temperature was recorded shortly after birth and at 24h. Cross-fostering was performed 24h after birth. Blood samples were obtained from 29 SP at day 21 to determine Insulin-like Growth Factor-I (IGF-1) level using the Mediagnost IGF-I ELISA E20 kit®. Total mortality and SP' mortality rate (percentage of SP piglets in the litter that died) were recorded during the first 24h of life and from day 1 to day 21. Data was analyzed using SAS 9.2. Litter was considered the statistical unit.
Results: After the first 24h, the total and SP' mortality rate was lower for the LIANOL group compared to the CONTROL group, respectively 2.1±1.8% vs. 7.1±1.1% (*P*=0.036) and 4.5±2.5% vs. 11.1±3.0% (*P*=0.047). From day 1 to 21, total and SP' mortality rate did not differ between groups, with respectively mean values 8.2±1.0% (*P*=0.634) and 14.9±2.4% (*P*=0.538). Piglets rectal temperature at 24h did not differ between groups with a mean value 38.2±0.1ºC (*P*=0.777). At day 21, SP' BW and average piglets BW did not differ between groups with respectively mean values of 3.73±0.06kg (*P*=0.395) and 5.7±0.2kg (*P*=0.268). The IGF-I levels in SP was higher in the LIANOL group compared to the CONTROL group, 138 vs. 100±6.7ng/ml (*P*=0.030).
Conclusion: These results suggest that oral supplementation with Lianol® Colostro might be effective to increase small piglets early survival (first 24h). Moreover, Lianol® Colostro increased small piglets' IGF-1 levels at day 21, which might have beneficial effects on their future growth.

Disclosure of Interest: None Declared

Keywords: mortality, oral supplementation, pig

Poster Abstracts

Welfare and Nutrition

PO-PT2-221

Effect of Isoquinoline Alkaloid on PRRS Immune Response in Weaning Pigs

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Introduction: Porcine Reproductive and Respiratory Syndrome virus (PRRSv) has been reported to negatively modulate pig immune response, particularly the inhibition of interferon-g (IFN-g) and increasing the number of interleukin10 (IL-10) producing cells. Thus, one of the strategies in controlling the immune-modulation of PRRSv is to use immuno-enhancer for PRRS immune response. Sangrovit Extra®, a plant extract from *Macleaya cordata*, mainly consists of isoquinoline alkaloids (IQs). The IQs induced biological activities; anti-inflammatory, anti-microbial and immunomodulatory activities. The purpose of this study was to investigate the effect of IQs on serum neutralizing (SN) antibody titer and specific IFN-g producing peripheral blood mononuclear cells (PBMC) to PRRSv in weaning pigs in Thailand.
Materials and Methods: Twenty four, 21-day-old, male piglets, bought from a PRRS-free herd, were individually housed and divided into 4 groups, (n=6 per group); A (non-vaccinated, no challenged), B (non-vaccinated, with challenged), C (vaccinated, with challenged) and D (vaccinated, 100 ppm of IQs with challenged) groups. The C and D pigs were vaccinated with the PRRS vaccine (Ingelvac PRRS® MLV, BI, Germany) at 28 days of age. The B, C and D pigs were challenged with 2 ml of field PRRSv 10^5TCID$_{50}$/ml and 10^7TCID$_{50}$/ml intramuscularly on day 28 and 42 post vaccination, respectively. Blood samples were collected on day 0, 28, 35, 42 and 49 for serum neutralizing antibodies and PRRS specific IFN-g producing PBMC, measured by flow cytometry (BD Accuri™ C6).
Results: The group D pigs (IQs 100 ppm) not only had the highest level of SN titer, in particular on D42 and D49, but also continuously increased percentage of specific IFN-g producing PBMC. Moreover, vaccinated pigs were found to have a higher level of both SN titer and the percentage of IFN-g producing PBMC than unvaccinated pigs. After the PRRSv challenge, percentage of specific IFN-g producing PBMC had increased drastically in vaccinated piglets compared to the slower response of SN titer.
Conclusion: Pigs fed Sangrovit Extra® supplemented feed enhanced both SN titer and specific IFN-g producing PBMC in PRRS vaccinated piglets compared to non-vaccinated pigs. In conclusion, the protective immune response to the PRRS infection should be focused more on IFN-g producing cells than the SN titer, particularly in vaccinated piglets.

Disclosure of Interest: None Declared

Keywords: phytobiotics, PRRS control, weaning pigs

Welfare and Nutrition

PO-PT2-227

Evaluation of the effects of maternal dietary application of seaweed extract (LactoShield®) on the performance of pigs

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Introduction: Supplementation of feed with seaweed extract has been shown to have a positive effect on performance, intestinal morphology, intestinal microflora and immune status in sows and post-weaned pigs. It was hypothesised that maternal seaweed extract (LactoShield®) supplementation from day 107 of gestation until weaning would enhance the growth performance of pigs.
Materials and Methods: Pregnant sows were fed with either 1) a basal lactation diet or 2) a basal lactation diet supplemented with LactoShield® from day 107 of gestation until weaning (28 days of age). The LactoShield® supplement was supplied by BioAtlantis Ltd. (Kerry, Ireland). Sows were individually fed and the supplement was added to a small initial portion of feed daily to ensure the full supplement was ingested. At weaning (28 days) the piglets were moved to separate houses according to their dietary treatment. There was a total of 67 piglets at weaning, basal group: n=33 and LactoShield® supplemented group: n=34. Feed and water was available to the piglets *ad libitum*. The amount of feed given to each group and the amount of waste feed removed was recorded daily. Piglets were weighed at birth and at 7, 11, 18, 26, 32, 40, 46, 53, 60, 67 and 78 days of age. Feed intake and body weights were used to calculate average daily gain (ADG) and gain to feed ratio (GFR). Analysis of variance was carried out on these data by ANOVA and by repeated measures analysis.
Results: Pigs from LactoShield® supplemented sows showed improved performance compared to those from sows on the basal diet. ANOVA analysis of variance demonstrated significantly increased ADG for the LactoShield® group between days 0-7, 25-32, 40-46, 53-60, 25-53 and 25-78 ($P<0.05$). A statistically significant higher average body weight was also observed in the LactoShield® treated group on days 7, 32, 46, 53, 60, 67 and 78 ($P<0.05$).
Conclusion: Assessment of performance parameters, demonstrated that pigs weaned from LactoShield® supplemented sows had a significantly improved ADG and average body weight. ADG was improved in the critical early period after weaning and from weaning to termination of the experiment at 78 days of age. While a trend towards increased GFR was observed between 40-46 and 53-60 days of age these data were not could not be confirmed statistically. These results are in line with other studies indicating that this supplement enhances gut health, immunity and performance in pigs. As such LactoShield® supplementation could be a useful management tool for pig producers in this post-antibiotic era and could help to improve margins in a difficult marketplace.

Disclosure of Interest: None Declared

Keywords: Feed supplement , Pig performance , Seaweed extract

Welfare and Nutrition

PO-PT2-231

Field study on fermentative profiles of swine liquid feed in Northern Italy

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Introduction: Liquid feed is the main feeding practice for finishing pigs in Northern Italy and is increasingly becoming a reference for sows and piglets (Lizardo, 2003). The objective of this field survey was to characterize the fermentative profile of different liquid feeds according to the main ingredients used in Northern Italy.
Materials and Methods: In order to monitor the fermentation profile of different types of liquid feeds used in fattening pigs in Northern Italy, and the possible effect of different ingredients on the fermentation, 75 samples of soup taken from 64 farms between March 2014 and November 2015 were analyzed. No samples were taken during the summer to avoid an extreme heat possible bias on fermentation. All samples were taken in the last phase of fattening (100-160 kg) and were tested for dry matter (DM), pH at sampling and 6 hours after, ethanol, volatile fatty acids (VFA: acetic acid, propionic acid) and lactic acid. Analyses were done on samples frozen after 6h. Soups were classified into 4 groups according to their composition besides water: dry feed (WATER), dry feed + high moisture corn (HMC), dry feed + whey (WHEY), dry feed + HMC + whey (HMC + WHEY). The addition of a lactic acid bacteria (LAB) based inoculant (*Pediococcus acidilactici* MA 18/5M) was also considered.
Results: pH of the soup at sampling and 6 hours later was the lowest ($P<0.001$) for WHEY diets. In addition, pH 6h was numerically lower when LAB was added, regardless of the components. WATER diets showed the lowest ($P<0.01$) ethanol level, and diets with whey showed the highest ($P<0.01$) lactic acid level. Acetic acid tended ($P<0.1$) to be the lowest in WATER diets. The addition of LAB had an effect on ethanol ($P<0.1$) and propionic acid ($P<0.05$) with the highest level of the two compounds when it was added. Lactic acid was numerically higher when LAB was added. A negative correlation ($P<0.01$) between lactic acid and pH after 6h, a positive correlation ($P<0.01$) between lactic acid and acetic acid, and a positive trend ($P<0.1$) between lactic acid and ethanol were identified, all the correlations regardless of the components. No year effect was depicted.
Conclusion: These results suggest that the composition of a liquid diet affect the quality of the feed. pH is strongly affected by the use of whey but it can be partially controlled by addition of LAB to ensure a better hygiene of the feed. Production of volatile fatty acids as a source of energy from the diet is also a positive effect of the addition of LAB. Mortality was not affected by diet profiles. The impact of soups composition on animal performances should be further studied on a larger number of farms.

Disclosure of Interest: None Declared

Keywords: Liquid feed, fermentation profile, lactic acid bacteria

Welfare and Nutrition

PO-PT2-237

Grouping of sows after weaning in a mixing pen did not reduce the level of lameness

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Introduction: According to Danish regulations gilts and sows must be housed in groups from weaning until 7 days before expected farrowing. Mixing sows both after weaning and after mating may/will impair reproduction results and increase prevalence of lameness. A Danish study shows that approximately 90 % of medical treatments in gestation are attributed to lameness.
The aim of the current study was to reduce the frequency of gilts and sows being treated for lameness in the period from weaning until three weeks after mixing in the gestation pen.
Materials and Methods: A total of 2350 gilts and sows in two herds were included in the study. In both herds, sows were weaned into a mating pen with an activity area with straw bedding and one free access stall per sow. At weaning sows were assigned to a control group were they had free access to the feeding stalls from weaning until transfer to the gestation unit and a test group where the sows were pre-mixed in the mating pen before transfer to the gestation unit. Mixing was done by refusing access to the stalls in brief periods of time. During mixing, the area per sow was 1.8 m^2 and 3.5 m^2 in herd 1 and 2, respectively. In the gestation unit gilt and sows were housed in stable groups and fed either in electronic sow feeding stations (herd 1) or long trough (herd 2).
The mixing strategies differed between herds. In herd 1, the transfer of sows into the gestation unit was done after insemination, whereas sows stayed in the mating pen for 4 weeks in herd 2 before transfer to the gestation unit. The sows were assessed for lameness in the gestation unit at week 0, 1, 2 and 3 on a scale of 1 to 4. Lameness was scored on a scale from 0 (healthy) to 4 (immobile).
Lameness was analysed using logistic regression in SAS, proc glimmixed, with group and weeks after mixing as fixed factors and batch*pen as random factor.
Results: In herd 1, the number of treated sows was significantly higher in the trial group compared to the control group ($p=0.0277$). There was no significant difference between the two groups in herd 2. Thus, mixing of sows under optimal conditions in the insemination unit did not have the expected effect. Possible explanations could be the smaller area per sow during mixing in herd 1, or the fact that the sows in herd 2 were loose in four weeks before pre-mixing in the mating pen. This is consistent with the treatment results, and indicates that the sows in herd 2 used the prolonged period of time in the insemination unit to complete establishment of the hierarchy.
Conclusion: In conclusion, the strategy where gilts and sows were weaned into a specially designed pen and pre-mixed failed to reduce the frequency of lame gilts and sows in the gestation unit.

Disclosure of Interest: None Declared

Keywords: Group housing, Lameness, Mixing pen

Poster Abstracts

Welfare and Nutrition

PO-PT2-238

Standardization of piglet euthanasia technique across a multiplication system

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Introduction: Euthanasia of pigs on farms is a common and inevitable process for the well-being of the animals. The American Association of Swine Veterinarians along with the American Veterinary Medical Association set guidelines for appropriate euthanasia techniques based on the size and age of a pig. Piglet euthanasia presents a considerable challenge due to the general lack of body fat, small size, and difficult handling. There are limited methods to effectively render a piglet insensible that are safe for the caretaker. Furthermore, the most common method of blunt force trauma (BFT) has variable efficacy dependent upon the caretaker and is aesthetically disturbing for many. The goal to cease BFT trauma within the PIC system was followed by a thorough literature and product review. The review narrowed the scope to two types of euthanasia: CO2 gas and non-penetrating captive bolt (NPCB) gun. Due to previous negative experiences with CO2 gas euthanasia, NPCB was determined as the method of choice for piglet euthanasia in the multiplication system within North America.
Materials and Methods: The Zephyr-EXL™ NPCB gun was the product purchased for the farms. Depending on the size of production at the site, at least two units were purchased (one main unit with a backup). Because the units are powered by compressed air, air compressors with a pressure gauge and capacity to reach 120psi were purchased for the farms. Keeping caretaker safety in mind, piglet restraints were constructed. The restraints were created using 10 inch PVC pipe that was cut longitudinally in half. The concave side of the halved pipe became the basin for restraining pigs. A key hole drill was used to create various sized holes for different sized piglet legs. The piglets are suspended in the basin of the pipe with the legs through the holes. A shoulder strap is pulled across the piglet's back to prevent movement. The pipe is secured to a feed cart via threaded screws. A hand shield is used to prevent caretaker injury.
Results: Since March of 2015, all of the multiplication system units have switched to NPCB euthanasia. Caretakers report 100% efficacy on the first attempt with the device when the air compressor is dialed to the correct pressure reading. The technique is effective regardless of the caretaker's strength.
Conclusion: In the interests of caretaker safety and well-being of the piglets in our care, BFT euthanasia was phased out on all multiplication system sites. NPCB euthanasia is the technique that replaced BFT. The new technique removes the caretaker needing a certain skill and strength to perform, and it has been well-received for its efficacy.

Disclosure of Interest: None Declared

Keywords: piglet euthanasia, welfare

Welfare and Nutrition

PO-PT2-245

The impact of different dietary levels of fermentable substrates and calcium-phosphate on the intestinal microbiota in pigs

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Introduction: Saving the limited resources of phosphate rock and improving intestinal health of pigs represent major challenges of modern pig industry. Consequently, the present study addressed the impact of supplementing variable levels of calcium-phosphate (CaP) on the numbers of selected members of the intestinal microbiome in growing pigs. To investigate the impact of fermentable substrates on the microbiota, two protein sources potentially providing variable quantities of fermentable substrates for the gut microbiota were utilized.
Materials and Methods: A total of 31 growing pigs (initial BW 54.7 kg ± 4.1 kg) in 2 consecutive experiments were allocated to 4 treatments, fed either a corn-soybean meal or a corn-pea based diet, each diet containing a high and a low level of CaP (Ca: 4.4 vs. 8.3; P: 4.2 vs. 7.5 g/kg dry matter) After 9 weeks, animals were slaughtered, digesta samples from jejunum, caecum, and colon were removed and 16S rRNA gene copy numbers of *Bifidobacterium* spp., *Enterobacteriaceae, Lactobacillus* spp., and *Roseburia* spp. were analyzed by quantitative real-time PCR.
Results: Low levels of CaP led to higher 16S rRNA gene copy numbers of all bacterial groups (P<0.10) except *Lactobacillus* spp. in the jejunum. In contrast, numbers of caecal *Roseburia* spp. were lower (P<0.05), when diets containing low CaP levels were fed, whereas colonic *Bifidobacterium* spp. (P<0.05) were higher. Higher quantities of *Roseburia* spp. were detected in the jejunum (P<0.05) when pea containing diets were fed, while feeding these diets resulted in lower numbers of this genus in the caecum (P<0.10). Jejunal *Lactobacillus* spp. (P<0.05) and caecal *Bifidobacterium* spp. (P<0.05) were lower, whereas *Enterobacteriaceae* were higher in all investigated gut compartments as a result of feeding pea containing diets (P<0.05).
Conclusion: In the present study, a reduction of dietary CaP resulted in higher numbers of bacteria with probiotic properties such as colonic *Bifidobacterium* spp. as well as higher numbers of jejunal *Enterobacteriaceae* including pathogenic members. Thus, an evaluation of potential effects of these diets on intestinal health is difficult. The utilization of pea meal instead of soybean meal containing diets led to higher numbers of *Enterobacteriaceae* in all investigated compartments, whereas probiotic *Lactobacillus* spp. and *Bifidobacterium* spp. were partly lower, indicating an assumed less favorable microbial composition. However, the observed effects cannot be assigned to P alone or to certain substrates because concentrations of Ca and P varied and diets contained different levels of various individual substrates.

Disclosure of Interest: None Declared

Keywords: None

Welfare and Nutrition

PO-PT2-247

Temporal changes in mechanical nociceptive thresholds in juvenile pigs subjected to surgical tail amputation: a model of injury induced by tail biting

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Introduction: Tail biting is a global welfare problem in the pig industry leading to significant tail injury and potential carcass rejection. The temporal effects of such injuries and subsequent healing are presently unknown, although limb amputation in humans can lead to abnormal neural activity and decreased nociceptive thresholds. In order to evaluate potential sensitisation following tail damage, we created a model by surgical amputation of tails, and assessed mechanical nociceptive thresholds.
Materials and Methods: Surgical tail resection was performed to assess the influence of age, extent of tail amputated and time since amputation on thresholds of mechanical nociception. To evaluate the effect of age at the time of injury, female pigs underwent surgery at 9 weeks (±3 days 'weaner') (n=19) or 17 weeks (±3 days 'finisher') (n=43). The effect of time after amputation was evaluated on 24 pigs at 8 weeks, and 38 pigs at 16 weeks after surgery. The effect of the extent of tail amputated was assessed by assigning the pigs to 3 treatments ('Intact': sham-amputation; 'short tail': 2/3 of tail removed; 'long tail': 1/3 of tail removed). A Pressure Application Measurement device was used to record mechanical nociceptive thresholds (tail flick or tail clamp withdrawal responses). Within a single session, three stimuli were applied to a skin area proximal to the site of amputation, 3 days pre-surgery, 1 week and either 8 or 16 weeks post-amputation.
Results: Across the two amputation ages, results indicated that tail amputation induced a significant reduction ($P<0.05$) in mechanical nociceptive thresholds in short and long tails one week after surgery. The same treatment effect was observed at 16 weeks after amputation performed at 9 weeks of age ($P<0.05$). For surgeries performed at 17 weeks of age, thresholds tended to be lower in short compared to intact tails ($P=0.081$) and significantly lower ($P<0.05$) in long tail pigs 8 weeks after amputation. No significant difference was observed at 16 weeks following surgeries performed at 17 weeks of age.
Conclusion: These results show that surgical amputation of pig tails leads to decreased cutaneous mechanical nociceptive thresholds in the skin area proximal to the site of injury. Results indicated that severe tail injury occurring in the weaner period may be associated with sensitisation up to 16 weeks following the injury. In contrast, injuries occurring in the finishing period appeared to be associated with shorter lasting mechanical sensitisation, resolving within 16 weeks.

Disclosure of Interest: None Declared

Keywords: , tail amputation, mechanical nociception, sensitisation

Welfare and Nutrition

PO-PT2-249

Effects of encapsulated products as alternatives for antibiotics and Zn on piglets' performance and intestinal mucosa

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Introduction: The use of alternatives to antibiotics, such as essential oils, organic acids and zinc oxide for the maintenance of swine health and performance, has been under debate. However, their efficacy varies due to many reasons and also to their availability in the intestine. Encapsulation methods have been applied to protect these compounds against gastric acidity and promote the gradual release to the distal parts of the intestine. The objective of the study was to evaluate the effects of the dietary addition of two encapsulated products on the performance of weaners, but also to test the hypothesis that the elevated quantities of acids, zinc oxide and antibiotics could be effectively replaced by such products.
Materials and Methods: A field study was conducted in a commercial swine farm of a capacity of 320 sows, in Greece. The design comprised three treatments in the post-weaning period (28 - 63 days of age): Treatment C (control); Treatment A, diets supplemented with Product A (encapsulated calcium formate, benzoic and citric acids); Treatment B, diets supplemented with Product B (as of product A and additionally supplied by encapsulated essential oils and encapsulated Zinc Oxide). Antibiotics were supplemented in the prestarter phase in all groups (days 28-42), while in the starter phase were supplemented only in groups C and A (days 42-63). Each treatment comprised 10 replicates (10 pigs/replicate). Performance parameters of pigs included body weight and feed consumption. At end of the study five (5) pigs per treatment were randomly selected in order to obtain Hematoxylin- Eosin stained sections of standardized jejunum and ileum areas for histopathological examination.
Results: Piglets from treatment A exhibited a higher daily growth rate from days 42 to 63 ($P=0.029$), which tended to be significant for the overall period ($P=0.064$). This resulted to a tendency for higher weight at the end of the trial, which was approximately 1.5kg more than the other 2 treatments ($P=0.058$). Piglets from treatment B had similar performance with the control group. The mucosal height of jejunum in pigs of groups A and B was significantly higher by comparison with that of control ($P=0.02$ and $P= 0.009$ respectively).
Conclusion: The obtained results here confirmed the initial hypothesis set, for the effective use of encapsulated alternatives in the post-weaning period in pigs. Moreover, piglets from B treatment, had similar growth with piglets fed the control treatment that contained antibiotics throughout the study.

Disclosure of Interest: G. Papadopoulos: None Declared, T. Poutahidis: None Declared, N. Tallarico Conflict with: Participation in study design, G. Arsenos: None Declared, P. Fortomaris: None Declared

Keywords: encapsulated products, growth, piglets

Poster Abstracts

Welfare and Nutrition

PO-PT2-250

Compounding iron dextran with meloxicam or flunixin meglumine for use in piglets at time of processing

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Introduction: Research has documented that non-steroidal anti-inflammatory drugs (NSAIDs) reduce pain and pain related behaviors in piglets. The use of analgesics at the time of piglet processing increases the amount of piglet handling and injections as well as increases the labor for the producer. The ability to give an injection of an NSAID at the same time as an iron product (compounded) would help minimize animal handling and labor. The objective of this study was to determine if the compounding of iron dextran (Ferroforte® 200 mg/mL) and the NSAID meloxicam (Metacam® Injectable Solution 20 mg/mL) or flunixin meglumine (Banamine® Sterile Solution Injectable 50 mg/mL) would reduce the amount of (NSAID and/or iron dextran) available to the piglet.
Materials and Methods: *Part 1:* Sixty piglets were selected at 2d of age and were assigned to 1 of 3 treatment groups: Iron dextran (ID), compounded ID and meloxicam, or compounded ID and flunixin meglumine. Prior to treatment a whole blood sample was taken at 3d of age to determine the baseline hemoglobin status of each piglet. A second blood sample was taken at 21d of age (just prior to weaning). Hemoglobin (Hgb) concentrations were analyzed in at the Animal Health Laboratory (AHL), University of Guelph. Mixed linear regression was used to examine the effect of treatment on Hgb levels just prior to weaning.
Part 2: Forty piglets were selected at 3-4d of age and were assigned to receive 1 of 5 treatments: ID alone, compounded ID and meloxicam, compounded ID and flunixin meglumine, meloxicam alone, and flunixin meglumine alone, given intramuscularly. Blood samples were taken at regular intervals over 72 hours via pre-placed jugular catheters. Plasma was analyzed using mass spectrometry methods to determine NSAID levels. Pharmacokinetic parameters for plasma meloxicam and flunixin meglumine concentration-time profiles were determined for each animal using noncompartmental pharmacokinetic analysis approaches.
Results: No significant effect of compounding either NSAID with ID on measured blood Hgb levels was found. Significantly reduced concentrations of both NSAIDs (Cmax and AUC) were found when compounded with ID compared to the levels noted when either NSAID was given alone.
Conclusion: The compounding of meloxicam or flunixin meglumine with ID produces a likely drug interaction, which does not appear to affect the ability of ID to maintain adequate Hgb concentrations, but does reduce the bioavailability of the NSAID for absorption into the systemic circulation. The clinical ramifications of these findings require additional efficacy studies to evaluate whether adequate analgesia is being provided at the current NSAID concentrations in the compounded formulation.

Disclosure of Interest: None Declared

Keywords: analgesia, NSAIDs, Pharmacokinetics

Welfare and Nutrition

PO-PT2-258

Good management routines around farrowing are essential for piglet survival in loose-housed sow herds

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Introduction: Piglet mortality, mainly within the first 4 days after farrowing, is still an important welfare issue and an economical challenge. Variation in piglet mortality between herds with similar genetic material and physical environment indicates that the herd`s management is a crucial factor. The objective of this survey was to assess the importance of different management routines around the time of farrowing and other farm qualities for piglet survival in loose-housed herds.
Materials and Methods: This field survey included 52 farms with hybrid sows of LY (Norsvin Landrace X Swedish Yorkshire). The farms were visited and farmers answered a questionnaire about their management practices. The outcome was the average herd pre-weaning mortality in the years 2012-2013. To include as many management factors as possible into the multivariable linear regression model, we generated a new variable based on 4 management routines; 3 routines at farrowing (presence at 80-100% of the farrowings, drying newborn piglets, split suckling), and one regarding farmers` contact with the sows. This variable was called “Management type” (M), and were divided into 4 categories with increasing effort; M1 herds (53.8%) without any of the 4 routines, but with others, M2 (21.2%) had contact with sows >2 times per day, M3 (17.3%) performed the 3 routines at farrowing, and M4 (7.7%) combined the contact and the 3 routines.
Results: Mean herd piglet mortality was 16.9±0.6% (5.5-28.3). The respective piglet mortality values for M1, M2, M3 and M4 herds were 18.6%, 15.6%, 15.1%, and 13.6%. When running the model and controlling for important confounders, the increasing management effort from M1-M4 was associated with lower piglet mortality ($P<0.05$).
Conclusion: We found piglet mortality to be multifactorial, as many management factors together lead to reduced mortality. Results in this study indicates that a high degree of farmer`s presence during farrowing, together with drying newborn piglets, split suckling and frequent contact with the sows, were important for reduced piglet mortality. Presence at farrowing makes the farmer able to discover individuals in need of attention, drying newborn piglets prevents them to become chilled, and split suckling secures colostrum to the smallest piglets in a litter. Frequent contact will probably reduce the sow`s level of fear and stress at farrowing, and is therefore positive for piglet survival. Overall, this study suggests that farmers with high management effort in the crucial period around farrowing, are credited for this work by getting a higher piglet survival.

Disclosure of Interest: None Declared

Keywords: loose-housing , management, pre-weaning mortality

Welfare and Nutrition

PO-PT2-259

Production results on the "Sow comfort" farrowing pen for loose housed sows

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Introduction: The objective of the present work was to collect preliminary production data on a newly-developed farrowing pen for individually loose-housed sows and to use these results to produce a pen for commercial practice which has a high piglet survival rate and improved sow and piglet welfare.
Materials and Methods: The "Sow Comfort" farrowing pen" (7.9 m^2) comprises two compartments: a "nest area" and an activity/dunging area with a threshold in between. The nest area has solid side walls, sloped walls on three of the sides and a hay rack on the fourth wall allowing free access to hay or straw. The nest area had two zones with floor heating covered by a 30 mm thick rubber mattress. Forty clinically health sows (28 Australian sows and 12 Norwegian, balanced for parity) were used in the first experiment. In a commercial farm, 350 Norwegian LY sows were tested in the system.
Results: The preliminary results show that the production results were similar to, or better than, those reported for other types of pens for individually loose-housed sows. Mortality of live born piglets was 12.1±2.9 % in the Norwegian sows and 12.9±2.0 % in the Australian sows whereas the number of weaned piglets in both countries was 12.1±0.4 and 9.1±0.3, respectively. Overlying was the most common causes of death and were significantly affected by parity and litter size. All sows showed a high level of communication with their piglets, and primiparous sows communicated significantly more with their newborns during the birth process than the pluriparous sows. At parturition, 33.3% of the sows were resting with the back towards the back wall whereas 41.7% rested towards the threshold. In 50% of the nursings, the sows were resting against the back wall while 30% of the nursings occurred towards the threshold. In a commercial version of this pen, without the threshold but with all other pen characteristics remaining, we ran around 350 LY-sows with different parities over 7 different batches through this pen system in one lactation. The results showed that piglet mortality declined in the first batch from around 15 % to below 12 percent in the 7^{th} batch, clearly showing that more experience with this pen system created really good production results and with a relatively small management effort by the farmer.
Conclusion: This pen system clearly stimulates the sow to perform better as mothers and releases the farmer from some of the management practices without impairing the production results. With an average og 14 liveborn piglets, these results shows that this pen is a promising alternative to other farrowing pens for loose-housed sows, and that it also can match the results from farrowing crates.

Disclosure of Interest: None Declared

Keywords: None

Welfare and Nutrition

PO-PT2-260

Ceva Valora® as an efficient alternative to surgical castration

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Introduction: Male pigs are castrated in order to limit or eliminate problematic aggressive behavior, uncontrolled mating and a boar taint, which represents a significant food quality problem. It is desirable for the welfare reasons to avoid surgical castration. Vaccination against boar taint utilizing the immune response to neutralize the endogenous LHRH (GnRH) is an efficient alternative which brings additional benefit of better feed efficiency. The aim of this study was to compare a new vaccine Ceva Valora® (Ceva) to a previously licenced product and assess the efficacy of a variable vaccination protocol.
Materials and Methods: Pigs were vaccinated with Ceva Valora (1ml s.c. – V4, V6, V8) or with the competitor product (2ml s.c., I4, I6, I8) at eight weeks of age, then a second injection was applied four, six or eight weeks before slaughter (at 26th week of age). Each group consisted of 10 pigs. The anti-LHRH antibody levels and the testosterone concentration were measured two weeks after booster vaccination and at slaughter time, while the concentrations of indole, skatole and androstenone were measured at slaughter time. Weight and size of testicles were assessed as one of the major efficacy criteria.
Results: Serological response to vaccination showed higher anti-LHRH titres in sera of pigs vaccinated with Ceva Valora® and a longer duration of high antibody titre than the ones obtained by the competitor vaccine. The concentrations of testosterone at slaughter were lower on average in Ceva Valora® groups than in the competitor vaccine groups, in which some individuals exceeded the levels >7 nmol/L. The concentrations of androstenone were below 0.04 mg/kg of fat in all pigs except one individual in I4 group with the concentration 0.54 mg/kg. This pig had also the biggest testicles and highest testosterone. The average testicle size in all groups vaccinated with Ceva Valora® was smaller than in competitor vaccine groups. The difference was statistically significant between groups V4/I4 for the length and for the weight.
Conclusion: The results indicate that vaccination with Ceva Valora® can result in highly efficient immunocastration. Compare to Improvac, Ceva Valora® had better performance in reducing the testicle size and weight and induce higher anti-LHRH antibody levels.

Disclosure of Interest: None Declared

Keywords: boar taint, Vaccination

Poster Abstracts

Welfare and Nutrition

PO-PT2-268

Feed trial comparing ADG of Ugandan pigs fed 3 diets: forage- or silage-based or commercial diets

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Introduction: Smallholder pig farmers in east Africa report that lack of feed, seasonal feed shortages, quality and cost of feed are key constraints to pig rearing. Commercially prepared pig diets are too expensive and there is competition for food between pigs and people. Smallholder farmers typically feed nutritionally unbalanced diets. This results in low average daily gain (ADG) and poor farmer profits. The objective was to compare the ADG of Ugandan pigs fed forage- or silage-based or commercial diets.
Materials and Methods: Local and crossbred Ugandan weaner-grower pigs were randomly assigned to commercial or forage- or silage-based diets. The forage-based diet, on an as-fed basis included specific amounts (%) of the following ingredients; avocado(25.5 kg), banana leaf(1.7), cottonseed meal(1.8), jackfruit(21.9), maize bran(9.5), sun-dried fish(3.1), sweet potato vine(36.1), limestone(0.14), salt(0.14) and vitamin/mineral premix(0.07). Silage-based diet included similar amounts of cottonseed meal, jackfruit, minerals and vitamins, but more maize bran (12.4), sun-dried fish(2.7), and ensiled sweet potato vine and tubers(60.8). Pigs were individually weighed every 3 weeks from 9 to 32 weeks of age. Pen-level ADG was compared across diets controlling for breed and starting weight using multiple linear regression.
Results: ADG of pigs fed commercial diet was higher than those fed forage- or silage-based diets between 9 and 24 wks of age($p<0.03$). Between 28 and 32 wks, pigs fed forage-based diets had a lower ADG than those on other diets ($P<0.001$). Least squares mean ADG (g/pig/day) for pigs fed commercial, forage- and silage-based diets were 294, 36, and 52 respectively at 9-15 wks; 329, 163, 212 at 15-19 wks; 574, 112, 362 at 20-24 wks and 1233, 694, and 994 at 28 to 32 wks of age.
Conclusion: Forage-and silage-based diets were unsuitable for newly-weaned pigs, which may be attributed to higher than anticipated diet ash and fiber contents. However, pigs on forage- and silage-based diets grew better than those on smallholder farms once they reached 20-24 wks and 15-19 wks respectively. This was when pigs were approximately 12 kg BW. Well-balanced cost-effective diets are needed to improve pig performance in east Africa. Fresh and ensiled locally available feedstuffs can be used in diets that meet the nutrient requirements of pigs. Low-cost forage- and silage-based diets containing some zero-cost feedstuffs are needed to improve the potential for profitability of smallholder pig farming. Efficient use of these feedstuffs is required to promote sustainable smallholder pig rearing enterprises.

Disclosure of Interest: None Declared

Keywords: Smallholder farmer, East Africa, forage- and silage based diets

Welfare and Nutrition

PO-PT2-270

INFRARED THERMOGRAPHY, BEHAVIOUR AND PERFORMANCE EVALUATION OF PIGLETS AFTER MELOXICAM ADMINISTRATION IN PRE CASTRATION PROCEDURE

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Introduction: Castration procedure is a painful method which reduces welfare and could reduce performance. Some trials demonstrated that meloxicam prior castration procedure in piglets could reduce acute pain decreasing behaviors relates to a lack of welfare. Any study using infrared thermography (IT) was found to evaluate the inflammatory process after castration procedure in piglets. This study aimed to evaluate inflammatory process using IT associated with behavior and performance in piglets treated or not with meloxicam 30 minutes before castration.
Materials and Methods: This trial was done in a commercial pig farm located at São Paulo State (Brazil). Ninety three (93) piglets were used from 21 sows (parity order 3.8±1.3) divided in 2 groups. Group 1 control (C) (n=46) were administered 0.1ml of saline solution and group 2 0.1ml of meloxicam (M) (n=47), (0,4mg/kg PV) (Maxicam® 2%, Ourofino Saúde Animal, Brazil). Both groups had an intramuscularly administration 30 minutes before castration. Infrared thermography (*Flir T-450sc, FLIR® Systems, Inc.*) was taken 3 times. The first (IT1) was took before castration (0h), second (IT2) 24h after castration and third (IT3) 48h after castration. All pictures were taken in the same position, scrotal area parallel to IT, in a distance of 0,40m. Behavior was analyzed 3 times, 0h, 24h and 48h after castration, with 10 minutes in each evaluation. Behavior evaluation included scratch (S) and tail wagging (TW). All piglets were randomly selected, identified, matched by similar weight and weighted individually 2 times, 0h and 48h after castration to evaluate average daily gain (ADG). Piglets were categorized in light (2.4–2.999kg) and heavy (3–4kg). All statistical analyses were done by SAS software. ADG and IT was analyzed by GLM model and behavior by FREQ procedure.
Results: There was no statistical significance for ADG and IT between C and M groups. Behavior showed statistical difference ($P<0.05$) in light piglets with higher frequency in TW in C than M group at 24h (81.48% x 54.83%, respectively – $p<0.03$) and 48h (81.48% x 51.61%, respectively – $p<0.01$).
Conclusion: Although IT evaluation couldn't demonstrate difference in inflammatory process in both groups, M showed better welfare behavior than C group, which indicates an anti-inflammatory action. Probably by the large amount of liquid due to edema at 24h in scrotal area incision, it was not possible to capture the temperature difference between groups. Further studies need to be done using IT in different moments to better associate inflammatory procedure with meloxicam effect, which in this study had a directly influence in behavior.

Disclosure of Interest: None Declared

Keywords: Infrared thermography, Meloxicam, piglets

Welfare and Nutrition

PO-PT2-272

STATISTICAL ANALYSIS OF THE BEHAVIOR OF MYCOTOXINS IN MEXICO DURING THE PERIOD 2010 TO 2014

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Introduction: Because of natural occurrence of mycotoxins in the finished food and grains used for the formulation of rations of various species, mycotoxicosis is considered a critical problem in swine feed production. Facing the difficulty of detection of mycotoxins in the finished food or raw material, it is important to increase the sampling frequency, because the effect of the mycotoxins is cumulative as the consumption of contaminated grains or contaminated food to reduce the risk of consumption.
Materials and Methods: There were used data of samples of food and raw materials submitted to Nutek Chemistry Laboratory, the samples were subjected to the detection of mycotoxins, the results were compiled from 2010 to 2014. During the period 2010-2014 there were performed a total of 30.509 testing to detect 12 types of mycotoxins: aflatoxins B1 (AFLAs B1), aflatoxin B2 (AFLAs B2), Aflatoxin G1 (AFLAs G1), aflatoxin G2 (AFLAs G2), ochratoxin A (okra), fumonisin B1 (Fumon B1), zearalenone (ZEA), deoxynivalenol (DON), T2 toxin (T-T2), diacetoxiscirpernol (DAS), nivalenol (NIV), neosolaniol (NEO) and citrinina (CIT). Data were analyzed statistically by the JMP program. It was used a completely randomized design and for the difference between means was used the Tukey test ($P < 0.01$). The detection method was performed for each mycotoxin had been done according to the provisions of the AOAC (high resolution liquid chromatography or gas chromatography-mass). The reference limits of mycotoxin contamination level based on the provisions of the European Union (2006/576 / EC, PRE / 1809/2006, 2013/165 / EU).
Results: According to the review period, the year of highest contamination was 2010 for the following mycotoxins: ochratoxin A, fumonisin B1 and deoxinivalenol ($P < 0.1$). Sorghum grain is mostly contaminated by ochratoxin A (3.07), while the DDGS and soybean meal was not contaminated by mycotoxin ($P < 0.01$), in the case of fumonisin B1, the DDGs raw materials were mostly contaminated by fumonisin B1 (637.80) while sorghum reported the lowest levels of contamination because of this mycotoxin ($P < 0.01$). About the grains referred for analysis, corn and DDGS samples are sent mainly to detection of mycotoxins.
Conclusion: In the analyzed period, 2010 was the most prevalent mycotoxin in the incidence of aflatoxin B1, deoxynivalenol, T2 toxin were reported with no significant statistically difference in all the raw materials analyzed. The main contaminated grain by Ochratoxin A is sorghum. The raw material mainly contaminated by fumonisin B1 are the DDGS. Aflatoxin B1 and deoxynivalenol found higher levels than those set by the European Union.

Disclosure of Interest: None Declared

Keywords: aflatoxin, deoxynivalenol, corn

Welfare and Nutrition

PO-PT2-279

Effect of dietary seaweed extract supplementation on the reproductive performance of sows and suckling pig performance.

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Introduction: Recent research has demonstrated that maternal dietary supplementation of LactoShield® (seaweed extract) had beneficial effects on the growth performance, immune status and gastrointestinal health of weanling pigs. The primary objective of the present study was to evaluate the effect of maternal dietary LactoShield® inclusion on the reproductive performance of sows and the piglet during the suckling period.
Materials and Methods: The farm used for this study was a commercial Truly Irish pig farm (Parknageragh Pig Breeders). In all, 138 crossbred pregnant sows (Large White x Landrace genetic lines) were randomly assigned accounting for parity and anticipated farrowing date, to 1 of 2 dietary treatments (n = 69 sows/treatment); (1) basal lactation diet and (2) basal lactation and LactoShield® from day (d) 109 of gestation until weaning (d 28). Upon farrowing, the numbers of pigs born alive, stillborn, and mummified were recorded. All pigs born alive were weighed and individually identified with numbered ear tags. At weaning, each litter was selected and all pigs within each litter were individually weighed. Data were analyzed using the GLM procedure (SAS Inst. Inc., Cary, NC) as a completely randomized design.
Results: The gestation length, number of pigs born alive, stillborn number, mummified number, average birth weight, total litter birth weight at birth, minimum birth weight and maximum birth weight within litters were not influenced by sow dietary treatment ($P > 0.05$). However, maternal supplementation of LactoShield® resulted in a significant reduction in the number of stillborn pigs (0.43 vs. 0.68; $P = 0.05$). The total litter weight at weaning was 5 % higher (94.55 vs. 90.22 kg) with the pigs from the LactoShield® supplemented sows when compared with the control group; however this effect was not significant ($P > 0.05$). Of particular interest, feeding LactoShield® during late gestation and lactation resulted in a significant reduction in the standard deviation (-9 %) and coefficient of variation (-10 %) of weaning weights within litters ($P < 0.05$). Consequently, supplementation of maternal LactoShield® resulted in a significant increase in the minimum weaning weight within litters when compared with the control group (5.86 vs. 5.47 kg; $P < 0.05$). In addition, the percentage of pigs weaned per sow on weaning day was significantly higher with LactoShield® supplemented sows when compared with the unsupplemented sows ($P < 0.01$).
Conclusion: The results of the present study show that LactoShield® supplementation reduced the number of stillborn pigs. In addition, LactoShield® supplementation decreased within-litter variation in piglet weaning weight.

Disclosure of Interest: None Declared

Keywords: Seaweed extract, Sow, Suckling piglet

Poster Abstracts

Welfare and Nutrition

PO-PT2-292

Assessment of the sow body condition in 46 Finnish commercial piglet producing herds

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Introduction: Sow body condition is a critical factor in all stages of the production cycle affecting health, welfare, performance and longevity. Additionally, the direct economic impact on annual costs of unoptimized feeding can be substantial. Therefore, it is important to monitor sows throughout their lifetime to ensure well-being, maximize production efficiency, optimize culling decisions and determine the adequacy of the feeding management practices. Multiple tools are available for the evaluation of the amount of body reserves, particularly muscle and fat. However, the methods differ considerably in reliability, comparability, simplicity and cost-effectiveness. Our aims were to study the health status using body condition scores (BCS) and backfat (BF) levels, and to measure the relationship between these variables using Finnish field data.
Materials and Methods: In total, 46 farms were enrolled in a cross-sectional, observational study, and visited once by the first author in 2014. Body condition measurements were taken from 26 – 67 females in every herd. Visual BCS was scored on a 1 (thin) to 5 (fat) scale. BF was measured in millimeters by the last rib (P2 method) using an ultrasonic instrument. The data of 2314 sows were scrutinized to describe the body condition traits, to tabulate their correspondence, and to assess the relationship using the Spearman's correlation coefficient.
Results: The results show, that the body condition status of the Finnish sows is at a favorable level. Majority of the sows (51.6 %) had an optimal condition score of 3, and only few individuals were classified as belonging to either the lowest (1.1 %) or the highest (1.1 %) category. BF levels ranged from 5 to 39, and 83.3 % of the sows had the BF of 10-22. Within each body condition class considerable variation in the BF was observed: 1 [5-13], 2 [6-22], 3 [8-27], 4 [12-33] and 5 [17-39]. The general association between the subjective BCS and the objective BF was moderately strong and significant (Spearman's rho=0.74, <0.0001), however it varied between farms (0.22-0.90).
Conclusion: The study suggests that in order to improve the precision and usefulness of the subjective BCS and to better monitor the fluctuations, the classical five-step rating scale should be divided into sub-categories. In practice, sow´s body condition should be individually evaluated, registered and managed on a regular and continuous basis to ensure appropriate nutrition and minimize herd variation. However, since BCS is not invariably a reliable method, the inclusion of the assessment of BF as an indicator of the fat reserves enhances the accuracy and comprehensiveness of the evaluation of the body condition and composition.

Disclosure of Interest: None Declared

Keywords: Body condition score, Correlation, P2 backfat depth

Welfare and Nutrition

PO-PT2-295

Effect of removal of antibiotics from the diet on the performance of negative behaviours in weaner pigs

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Introduction: To evaluate the effects of removing antibiotics (AB) from the diet of 1st and 2nd stage weaner pigs on the performance of negative behaviours.
Materials and Methods: Weaned pigs in a total of 12 groups of 35 pigs each, had in-feed AB removed (NO; n=6) or maintained in the diet (AB; n=6). Groups were observed 1 day/wk between 0900 and 1300h for 3x5min periods each and all occurrences of fights, head knocks, tail, flank and ear biting behaviour were recorded. Additionally, room temperature (RT) and CO_2 concentrations were measured once/wk. Body weight (BW) was recorded when pigs were weaned (i.e. moved into the 1st weaner stage accommodation) and on transfer to the 2nd stage weaner accommodation where groups were split and housed in 24 groups of c. 17 pigs each (AB; n=12 and NO; n=12). Behaviour observations were conducted in a similar manner except that groups were observed for 3x2.5min periods. To account for these changes data from the 1st and 2nd stages were analysed separately. Pen was the experimental unit. Data were analysed using linear mixed model equation methods. Stocking density, BW, RT and CO_2 were included as linear covariates. Group within treatment was included as a random effect.
Results: Treatment had no effect on tail and flank biting during both stages ($P > 0.05$). In the 1st stage, both ear biting (21.4 ± 2.15 vs. 17.3 ± 1.61 no./5min; $P < 0.05$) and the no. of fights (6.91 ± 0.91 vs. 5.58 ± 0.72; $P=0.09$) were higher in AB than in NO pigs. The frequency of tail biting increased with time in the 1st stage ($P < 0.05$). The no. of fights increased from the 1st to the 2nd wk and reduced in the following wks ($P < 0.05$). During the 2nd stage, the no. of fights and head knocks increased with time ($P < 0.05$). Ear, tail and flank biting increased from the 1st to the 3rd wk and reduced in the last week ($P < 0.05$). In pens with a higher frequency of flank biting during the 1st stage the stocking density ($P < 0.05$) and RT ($P < 0.05$) was higher. Additionally, pigs in pens where a higher frequency of ear biting was observed were heavier ($P < 0.05$). There was an increase in the no. of fights and incidences of ear biting with every 1ºC increase in RT during the 1st stage ($P < 0.05$); however, the opposite result was observed during the 2nd stage ($P < 0.05$).
Conclusion: Removing AB from pigs' diet during the weaner stages had a minimal effect on the performance of negative behaviours. The lower no. of fights and ear biting in NO pigs could be associated with reduced competition for access to feed arising from lower growth rates in these animals. This is supported by the fact that more ear biting was observed in pens where pigs were heavier.

Disclosure of Interest: None Declared

Keywords: Antibiotics behaviour diet

Welfare and Nutrition

PO-PT2-296

EFFECT OF THE NUTRITIONAL SUPPLEMENT VIUSID vet ON THE PRODUCTIVE BEHAVIOR AND THE HEMOGRAM OF REPLACEMENT SOWS

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Introduction: The productive behavior of the replacement sows should be taken into consideration. A good preparation of the future breeders is reflected in the cost, since managing them inappropriately may lead to 30% or more nonproductive days. The objective of this essay was to assess the effect of the nutritional supplement VIUSID vet on the productive behavior and hematology of replacement sows.
Materials and Methods:
For this work 64 Yorkshire sows of 97 days of age were used; 38 of them were treated and 26 remained as control group. The treatment consisted on providing VIUSID vet in a daily dose of 2 g/kg of concentrated food, during 70 days.
VIUSID vet contains: Malic acid, Glucosamine, Arginine, Glycine, Ascorbic Acid, Folic Acid, Monoammonium Glycyrrhizinate (extracted of the root of *Glycyrrhiza glabra*), Pyridoxine Hydrochloride, Cyanocobalamin, Calcium Pantothenate and Zinc Sulfate. The product undergoes a biocatalytic process of molecular activation to improve their biological activity and the biochemical reactivity of all their molecules.
Because of the existence of significant differences (P<0.05) among the initial weight of the two groups, the increment of weight was adjusted according to the effect of the initial weight as covariant. For the hematological study, samples of 26 sows were taken from the beginning to the end of the experiment (12 treated and 14 as control).
The dependent variables were: Initial weight, Weight gain, Daily mean gain, Alimentary efficiency, Initial hematocrit, Final hematocrit, Initial plasmatic protein, Final plasmatic protein, Initial leukocyte count , Final leukocyte count, Increment of hematocrit, Increment of leukocytes and Increment of plasmatic protein.
The test T was used for same or unequal variances, according to Levene test. For the pre-study and post-study of related samples, the T test for related samples was used.
Results: The results obtained in the productive behavior and in the hematological study, show that VIUSID vet significantly influenced (P<0.05) the weight gain (45.78 vs. 48.85 kg), the daily mean gain (0.654 vs. 0.698 kg) and the alimentary efficiency (3.91 vs. 3.66). It acted similarly on the increment of hematocrit (3.64 vs. 6.25 x 10^{-2} l/l) and on the increment of plasmatic protein (-0.09 vs. 0.24 g/dL). The other variables did not differ statistically.
Conclusion: Adding 2 grams of dietetic supplement VIUSID vet per kg of concentrated food, improved both the productive behavior and the hematologic values of replacement sows.

Disclosure of Interest: None Declared

Keywords: Molecular activation, sows, VIUSID

Welfare and Nutrition

PO-PT2-297

Effect of removal of antibiotics from the diet on welfare indicators of weaner pigs

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Introduction: The objective of this study was to evaluate the effects of removing antibiotics (AB) from the diet of 1st and 2nd stage weaners on welfare indicators.
Materials and Methods: The study involved a total of 12 pens of 35 pigs each where in-feed antibiotics were either removed (NO) or maintained in the diet (AB) during the 1st and 2nd weaner stages (9 weeks in total). Ten focal pigs were randomly selected from each group (AB, n = 60 and NO, n = 60). Limb lesions (i.e. calluses and swellings) were recorded as present or absent. Skin lesions in 11 regions of the pig's body were scored according to severity on a weekly basis and summed to give a total skin lesion score. Tail (TL, 0 to 5), ear (EL, 0 to 3) and flank (FL, 0 to 3) lesions were scored according to severity. Body temperature (BT) was also measured at each inspection. Pigs were weighed at weaning (i.e. on transfer into the 1st stage weaner accommodation) and on transfer to the 2nd stage weaner accommodation. Skin lesions were analysed using generalised mixed model equation methods. Tail lesions were re-classified as a binomial variable where 1 = presence and 0 = absence and along with limb lesions were analysed using binomial logistic regression. For all the studied variables, models included treatment, sex, inspection time and their interactions as fixed effects. Lesion scores at the start of the trial, stocking density, body weight and BT were included as linear covariates. Pig within inspection time was included as a random variable.
Results: Treatment and sex were not a significant source of variation for any of the variables studied ($P > 0.05$). Skin lesion scores increased as time progressed ($P < 0.05$) and pigs were also more likely to have calluses and swellings on the limbs during the subsequent weekly inspections compared to when the trial started ($P < 0.05$). Pigs were less likely to have tail lesions as time progressed compared to the first week on trial ($P < 0.05$). Higher stocking densities were related to higher body lesion scores ($P < 0.05$). Heavier pigs had higher skin lesion scores and were more likely to have swellings on the limbs ($P < 0.05$). Pigs with higher skin lesion scores also had higher BT ($P < 0.05$).
Conclusion: Removing antibiotics from the diet of weaner pigs had no effect on lesions related to pig welfare. However, variables such as time, stocking density and body weight seem to have an influence on welfare lesions.

Disclosure of Interest: None Declared

Keywords: antibiotics welfare weaners

Poster Abstracts

Welfare and Nutrition

PO-PT2-298

Phytogenic feed additive's efficacy in a protein-reduced diet in growing/finishing pigs

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Introduction: The experiment was conducted to evaluate the efficacy of a phytogenic feed additive (PFA) on body weight gain, carcass composition and quality of 72 finishing pigs [(Large White x Landrace) x Pi€train)].
Materials and Methods: Animals were distributed into 3 treatment groups considering litter, live weight and sex. The 3 treatment groups were control group (CON), phytogenic group 1 (PFA+normal) and phytogenic group 2 (PFA+low). The diet was based on corn, barley and soybean meal. CON and PFA+normal (Digestarom€ Finish 150 ppm, BIOMIN Phytogenics GmbH, Germany) received a diet containing 17% and 15% CP in the growing and finishing period, respectively. PFA+low (Digestarom€ Finish 150 ppm, BIOMIN Phytogenics GmbH, Germany) received a ration with a reduced protein content (-0.4% CP) throughout the whole growing and finishing period. Feed and water was provided ad libitum. At 73.2€0.6 kg body weight, feed changed from growing to finishing diet. Body weight was measured weekly and individual feed intake was recorded daily. Pigs were slaughtered at 117.5€0.2 kg and carcass composition and quality were evaluated. Data were analyzed with procedure GLM (SAS 9.4) and a multiple comparison was conducted with Tukey's range test.
Results: Results for the whole finishing period showed a significantly shorter finishing period (p
Conclusion: Overall, the supplementation of a PFA improved the performance of pigs and a protein reduction has been compensated efficiently through the supplementation of PFA.

Disclosure of Interest: None Declared

Keywords: finishing pigs, phytogenic feed additive, protein-reduction

Author Index

Author Index

Author Index

Author Index

Author Index

Author Index

Author Index

Author Index

Author Index

Author Index

Last Name	First Name	Abstract	Page
Holling	Carolin	PO-PT2-156	637
Hollis	William	PO-PF3-227	234
		PO-PW1-085	590
Holmes	Mark A.	PO-PF3-001	271
		PO-PF3-074	270
Holmgren	Sif	PO-PW1-087	578
Holtkamp	Derald	PO-PT2-207	322
		PO-PF3-112	471
		PO-PF3-182	459
Holtslag	Hans	PO-PT2-127	502
		PO-PT2-137	494
Hölzle	Ludwig	PO-PT2-245	648
Homuth	Matthias	PO-PF3-197	178
Homwong	Nitipong	PO-PC03-006	236
		PO-PF3-024	453
		O-MYC-001	166
Hong	Jin Su	PO-PT2-042	616
		PO-PT2-187	642
Honorato Gatto	Igor Renan	PO-PF3-249	203
		PO-PF3-151	260
Hoofs	Anita	PO-PW1-240	382
Hoogland	Marlin	PO-PW1-086	584
		O-IV-002	160
Horgan	Karina	PO-PW1-280	282
Hostnik	Peter	PO-PF3-110	452
Houben	Manon	PO-PW1-041	525
		PO-PW1-042	524
		PO-PW1-240	382
		PO-PT2-283	336
		O-RES-009	133
Houe	Hans	PO-PT2-035	498
HOULBERT	Jérôme	PO-PF3-153	252
Hsieh	Ming-Wei	PO-PF3-199	419
Hu	Ruiming	PO-PF3-065	451
Hua	Chuanxian	PO-PT2-244	500
Huang	Chienjin	PO-PF3-144	414
Huang	Lv	PO-PW1-164	561
		PO-PW1-146	560
		PO-PW1-187	564
Huang	Qinfeng	PO-PW1-176	566
Huang	Rose	PO-PF3-131	193
Huang	Tien Shine	PO-PW1-044	533
Huang	Weng-Zeng	PO-PF3-218	424
		PO-PF3-237	428
		PO-PF3-199	419
Huang	Yu Liang	PO-PW1-044	533
Huber	Mathias	PO-PW1-017	355
HUGUES	Laure	O-VET2-007	125
		PO-PT2-045	294
Hui	Raymond Kin-Hi	PO-PW1-122	559
Hulme	Scott	O-HHM2-007	151
Hulst	Marcel	PO-PT2-067	621
Hultén	Cecilia	PO-PW1-270	275
Hume	Michael	PO-PCO1-016	279
Hunger	Christine	PO-PT2-298	656
Hur	Jin	PO-PF3-100	410
		PO-PF3-210	423
Hüther	Liane	PO-PT2-149	635

Last Name	First Name	Abstract	Page
Hutjens	Madelon	PO-PW1-129	562
		PO-PT2-084	300
Hyun	Soobin	PO-PT2-183	476
Ibanez	Antonio	PO-PF3-180	227
Ibanez	Gabriela	PO-PF3-244	430
		PO-PF3-236	428
		PO-PF3-049	261
Ibañez	Ana Maria	PO-PF3-099	174
Ibar	Mariela	PO-PF3-143	226
Ibelli	Adriana M. G.	PO-PW1-011	352
Ikeda	Keiko	PO-PW1-166	592
Ikezawa	Mitsutaka	PO-PW1-181	567
		PO-PW1-101	593
Imbault	Heloise	PO-PF3-044	404
In Ho	Kim	PO-PT2-141	633
		PO-PT2-144	633
Infantes-Lorenzo	José A	PO-PF3-019	262
Ingrid	Rodrigues	PO-PC03-007	289
Inoue	Ryo	PO-PT2-011	344
		PO-PW1-132	555
Irvine	Claire	PO-PW1-010	352
Iscaro	Carmen	PO-PT2-081	446
Iseki	Hiroshi	PO-PW1-166	592
Ishikawa	Hiromichi	PO-PW1-188	595
		PO-PW1-166	592
Ishizeki	Sayoko	PO-PW1-188	595
		PO-PW1-166	592
Islam	Md. Aminul	PO-PW1-196	535
Ivanova	Stanislava	PO-PF3-301	215
		PO-PF3-240	194
Izquierdo	Mercedes	PO-PT2-088	623
Jablonski	Artur	PO-PF3-193	189
		PO-PF3-101	209
		PO-PF3-031	264
		PO-PF3-055	175
Jabłoński	Artur	PO-PF3-029	198
Jackova	Anna	PO-PF3-176	453
Jacobs	Amy	PO-PCO1-012	274
		PO-PT2-024	503
Jacobson	Magdalena	PO-PT2-096	625
		PO-PF3-060	270
Jaeger	Friedhelm	PO-PT2-089	623
Jaganathan	Seetha	PO-PW1-253	369
		PO-PW1-256	371
Jagu	Remy	PO-PT2-241	329
Jaime	Jairo	PO-PT2-253	475
		PO-PT2-204	474
		PO-PCO1-003	530
Jakobsen	Alex Stricker	O-PA-001	163
		PO-PF3-005	363
Jakobsen	Sine Stricker	O-PA-001	163
		PO-PF3-005	363
Jakubowski	Tadeusz	PO-PCO1-010	534
Jamawat	Supachai	PO-PW1-251	389
		PO-PF3-220	425
Jame Stott	Christopher	PO-PW1-056	514
Janas-Martindale	Alicia	O-VVD5-016	157
		O-VVD5-017	159
Janczak	Andrew M	PO-PT2-108	627

Author Index

Author Index

Author Index

O = Oral | **PO** = Poster | **PW1** = Posters displayed on Wednesday 8th June
PT2 = Posters displayed on Thursday 9th June | **PF3** = Posters displayed on Friday 10th June
PO-PC01 = Poster Corner displays for Wednesday 8th June | **PO-PC02** = Poster Corner displays for Thursday 9th June
PO-PC03 = Poster Corner displays for Friday 10th June

Last Name	First Name	Abstract	Page
Li	Yongtao	PO-PW1-094	533
		O-VVD2-009	110
		PO-PW1-118	534
Liang	Wan	PO-PF3-030	262
Liao	Chi-Ming	PO-PF3-144	414
		PO-PF3-201	420
Liboreiro Brustolini	Ana Paula	PO-PW1-216	370
Lieber	Anna-Karin	PO-PT2-293	338
Lien	Ying-Xiu	PO-PF3-253	463
Liesegang	Annette	PO-PT2-145	634
Lietz	Georg	PO-PT2-150	635
Lillie-Jaschniski	Kathrin	PO-PT2-057	604
		PO-PF3-142	211
Lim	Choon Kwang	PO-PF3-253	463
Lim	Jeonggyo	PO-PCO1-011	509
Lim	Changwon	PO-PT2-275	503
Limpiwattakee	Peerawan	PO-PW1-210	379
Lin	Jiunn-Horng	PO-PF3-218	424
Lin	Boh Chang	PO-PF3-053	244
Lin	Chao-Nan	PO-PW1-198	537
		PO-PF3-253	463
		PO-PF3-306	243
		PO-PW1-123	547
		PO-PF3-154	218
		PO-PW1-077	513
Lin	Cui	PO-PT2-185	474
Lin	Hui-Jei	PO-PF3-218	424
		PO-PF3-237	428
		PO-PF3-199	419
Lin	Jiunn-Horng	PO-PF3-237	428
		PO-PF3-199	419
Lin	Wei-Hao	PO-PW1-123	547
		PO-PF3-154	218
Lin	Ya-Ling	PO-PF3-253	463
Lin	Yixiao	PO-PT2-244	500
Lincopan	Nilton	PO-PF3-194	188
Lind	Peter	PO-PF3-097	174
		PO-PW1-133	591
Lindahl	Erik	PO-PW1-270	275
Lindberg	Ann	PO-PT2-147	312
Lindner	Thomas	PO-PF3-142	211
Linhares	Daniel	PO-PF3-246	239
		PO-PF3-047	462
		PO-PT2-037	292
		PO-PCO1-004	517
		PO-PF3-112	471
		PO-PW1-073	519
		O-VVD1-003	109
		PO-PF3-182	459
Linhares	Daniel C.	O-VVD2-006	111
Lino Fiúza	Aparecida Tatiane	O-REP2-007	118
Lippke	Ricardo	PO-PF3-280	244
Lisboa	Nazaré	PO-PW1-204	380
Lisgara	Marina	O-REP3-011	120
Liu	Guangliang	PO-PW1-053	510
Liu	Guoquan	PO-PW1-124	585
Liu	Hsiao Tau	PO-PT2-224	324
Liu	Huan	PO-PW1-176	566
Liu	Huili	PO-PW1-062	530

Last Name	First Name	Abstract	Page
Liu	Shun-Ren	PO-PT2-224	324
Liu	Zhengfei	PO-PF3-065	451
Liudmyla	Dudar	PO-PT2-282	486
Lizano	Sergio	PO-PW1-108	558
Lo	Dan-Yuan	PO-PF3-105	269
Lobato	Zélia	PO-PT2-119	608
Lobos	Maria Paz	PO-PF3-125	183
Loebert	Sandra	PO-PW1-275	276
Loeffen	Willie	PO-PT2-094	600
Loesken	Svenja	O-VET2-009	126
		O-RES-008	132
Lohse	Louise	PO-PW1-079	521
Loitsch	Angelika	PO-PT2-058	449
Loneragan	Guy	PO-PCO1-016	279
Looft	Christian	PO-PF3-215	423
Lopes	Pedro	PO-PW1-157	583
Lopes Antunes	Ana Carolina	PO-PW1-128	542
Lopes Mechler	Marina	PO-PF3-151	260
Lopez	Aurelie	PO-PT2-303	347
		PO-PW1-248	376
		PO-PF3-103	411
Lopez	Jose Antonio	PO-PT2-229	478
LOPEZ	Sebastien	PO-PW1-109	573
López	Walter Ricardo	PO-PW1-222	381
López	Will	PO-PW1-138	588
López Soria	Sergio	PO-PT2-243	476
Lopez-Rincón	Gonzalo	PO-PF3-184	192
López-Soria	Sergio	PO-PF3-229	232
Lorencova	Alena	PO-PW1-027	360
Lorgeré	Jean Claude	PO-PF3-221	246
Lorini	Dante	PO-PF3-152	414
Losio	Nadia	PO-PW1-298	444
Loureiro Bracarense	Ana-Paula	PO-PT2-091	624
Loving	Crystal	PO-PW1-131	552
		O-BBD1-001	134
Lowe	Erin	PO-PC03-018	291
		PO-PT2-068	298
Lowe	James	PO-PW1-085	590
Lozada	Maria Ines	PO-PT2-198	609
Lozada	María Ines	PO-PF3-143	226
Lozano	A M	PO-PW1-036	521
Lozano	Bernardo	PO-PW1-067	526
Lozano	Jose Maria	PO-PW1-010	352
Lü	Xinhui	PO-PW1-124	585
Lucia Jr.	Thomaz	PO-PW1-242	383
		PO-PW1-243	384
Ludwig	Carolin	PO-PF3-107	245
		PO-PF3-010	197
Ludwig Takeuti	Karine	PO-PF3-293	212
Luebbe	Jeff	PO-PW1-081	531
Luehrs	Adrian	PO-PC03-009	240
		PO-PW1-260	398
		O-MYC-002	166
		PO-PF3-260	431
Lugsomya	Kittitat	PO-PW1-297	443
		PO-PF3-296	207
Luís Guilherme	Oliveira	PO-PT2-294	339
Luna	Uanderson	PO-PT2-059	619

Author Index

O = Oral | **PO** = Poster | **PW1** = Posters displayed on Wednesday 8th June
PT2 = Posters displayed on Thursday 9th June | **PF3** = Posters displayed on Friday 10th June
PO-PC01 = Poster Corner displays for Wednesday 8th June | **PO-PC02** = Poster Corner displays for Thursday 9th June
PO-PC03 = Poster Corner displays for Friday 10th June

Last Name	First Name	Abstract	Page
Maragkakis	Georgios	PO-PW1-193	558
Marante	Rodney	PO-PF3-126	412
Marca-Puig	Joan	PO-PT2-155	313
		PO-PF3-299	198
		PO-PF3-124	202
		PO-PF3-163	201
March	Ricard	PO-PF3-045	404
		PO-PF3-254	430
Marchal	Leon	PO-PF3-258	231
		PO-PW1-129	562
		PO-PT2-061	620
		PO-PT2-084	300
		O-RES-005	131
Marchand	Dominique	PO-PT2-087	301
Marco	Terry	PO-PF3-053	244
Mariani	Jacopo	PO-PCO1-014	286
Marín-Alcalá	Clara Mª	O-VET3-012	127
Markowska-Daniel	Iwona	PO-PT2-022	604
Marois-Créhan	Corinne	O-VVD2-007	111
Marques	Nuno	PO-PF3-273	205
Marshall	Frank	PO-PW1-084	572
		O-VVD4-001	157
Marsteller	Thomas	PO-PF3-150	201
Martelli	Francesca	PO-PCO1-019	284
Martelli	Paolo	PO-PW1-122	559
		PO-PW1-021	357
Martens	Marc	PO-PF3-068	454
		O-WN1-002	141
		PO-PC02-011	610
Marthaler	Douglas	PO-PW1-065	510
		PO-PF3-024	453
		PO-PCO1-002	458
		O-VVD1-005	109
Marti	Hanna	PO-PF3-235	366
Martineau	Henny M.	PO-PT2-263	487
MARTINELLI	Nicola	PO-PF3-181	417
Martinez	Adam	PO-PW1-220	391
Martinez	Jorge	PO-PW1-057	528
Martinez	Rebeca	PO-PT2-190	607
Martinez	Xochitl	O-HHM3-012	153
Martínez	Adam	PO-PT2-051	598
Martínez	Atalo	PO-PW1-159	557
Martínez	Simon	PO-PW1-159	557
Martinez-Lobo	Javier	PO-PW1-100	540
		PO-PW1-098	541
Martinez-Lopez	Beatriz	PO-PT2-068	298
Martinez-Macipe	Miriam	PO-PT2-088	623
Martínez-Moreno	Álvaro	PO-PW1-287	438
Martins	Simone M. M. K.	PO-PT2-210	322
Martins de Souza	Dione	O-REP2-007	118
Junior		PO-PW1-216	370
Martos	Alba	PO-PF3-183	417
Masarikova	Martina	PO-PF3-129	203
Maskell	Duncan	O-BBD1-001	134
Maskell	Duncan J.	O-BBD1-005	134
		PO-PF3-001	271
		PO-PF3-074	270
Matajira	Carlos	PO-PF3-194	188
		O-BBD1-004	135

Last Name	First Name	Abstract	Page
Mateu	Enric	O-RES-002	129
Matheson	Stephanie	O-WN1-001	141
Mathieu	Christian	PO-PW1-095	555
Mathur	Pramod	PO-PC02-011	610
Matiasovic	Jan	PO-PF3-123	411
Matthijnssens	Jelle	PO-PF3-114	455
Matthijs	Anneleen	PO-PT2-040	615
Matyba	Piotr	PO-PT2-302	483
Matzinger	Shannon	PO-PT2-034	491
		PO-PT2-003	486
		O-VVD4-002	156
Mawhinney	Ian	PO-PW1-272	275
Maxwell	Charles	PO-PC02-006	205
Mayer	Katharina	PO-PT2-145	634
Mayerhofer	Sophie	PO-PF3-242	186
Mayrhofer	Sigrid	PO-PF3-298	191
Mazerolles	Philippe	PO-PT2-260	651
		PO-PT2-264	332
		PO-PF3-103	411
		PO-PW1-021	357
Mazzoni	Claudio	PO-PF3-295	250
		PO-PF3-287	250
		PO-PW1-258	375
McAuliffe	Mike	PO-PT2-279	653
McCabe	Lorna	PO-PF3-085	456
McCann	Ryan	PO-PW1-085	590
McDonnell	Mary J.	PO-PW1-276	283
McElroy	Máire	PO-PT2-002	254
		PO-PW1-010	352
McGettrick	Shane	PO-PT2-002	254
		PO-PW1-010	352
McKay	Karen	PO-PF3-085	456
McKeith	Floyd K.	O-MIS-004	170
McKenna	Stephen	O-VET1-004	123
McKillen	John	PO-PF3-114	455
		PO-PF3-128	464
		PO-PF3-085	456
		PO-PT2-200	482
		PO-PT2-227	646
		O-MIS-005	171
McLean	Allen	O-MIS-003	170
McLernon	Joanne	PO-PW1-284	437
McMenamy	Michael	PO-PT2-200	482
McNeil	Mark	PO-PF3-006	261
Mead	David	PO-PT2-009	482
Mechler	Marina L.	PO-PF3-259	466
		PO-PT2-201	606
Mechler	Marina Lopes	PO-PW1-026	360
Medeiros	Andrea S. R.	PO-PF3-256	465
Medina	Juan Carlos	PO-PT2-044	617
Medina	Marta	PO-PW1-189	543
Medina	Rafael	PO-PT2-007	599
Medina	Raphael Mansur	PO-PF3-042	267
Meedom	Leif	PO-PF3-302	193
		PO-PT2-197	643
		PO-PW1-278	281
Meens	Jochen	O-BBD3-012	139
Megens	Suzan	PO-PT2-283	336
Meijer	Wim	PO-PF3-020	259

Author Index

Author Index

Author Index

Author Index

Author Index

Author Index

Author Index

Author Index

Author Index

Author Index